VOLUME 35 APRIL 1957 NUMBER 4

Canadian Journal of Chemistry

Editor: LÉO MARION

Associate Editors:

HERBERT C. BROWN, *Purdue University*
A. R. GORDON, *University of Toronto*
C. B. PURVES, *McGill University*
Sir ERIC RIDEAL, *Imperial College, University of London*
J. W. T. SPINKS, *University of Saskatchewan*
E. W. R. STEACIE, *National Research Council of Canada*
H. G. THODE, *McMaster University*
A. E. VAN ARKEL, *University of Leiden*

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF CHEMISTRY

(Formerly Section B, Canadian Journal of Research)

Under the authority of the Chairman of the Committee of the Privy Council of Scientific and Industrial Research, the National Research Council issues THE CANADIAN JOURNAL OF CHEMISTRY and six other journals devoted to the publication, in English or French, of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; and members representing the Royal Society of Canada and four other scientific societies.

The Chemical Institute of Canada has chosen the Canadian Journal of Chemistry and the Canadian Journal of Technology as its medium of publication for scientific papers.

EDITORIAL BOARD

Representatives of the National Research Council

A. N. Campbell, *University of Manitoba*
G. E. Hall, *University of Western Ontario*
H. G. Thode, *McMaster University*
D. L. Thomson, *McGill University*
W. H. Watson (Chairman), *University of Toronto*

Editors of the Journals

D. L. Bailey, *University of Toronto*
T. W. M. Cameron, *Macdonald College*
H. E. Duckworth, *McMaster University*
K. A. C. Elliott, *Montreal Neurological Institute*
G. A. Ledingham, *National Research Council*
Léo Marion, *National Research Council*
R. G. E. Murray, *University of Western Ontario*

Representatives of Societies

D. L. Bailey, *University of Toronto*
Royal Society of Canada
T. W. M. Cameron, *Macdonald College*
Royal Society of Canada
H. E. Duckworth, *McMaster University*
Royal Society of Canada
Canadian Association of Physicists
K. A. C. Elliott, *Montreal Neurological Institute*
Canadian Physiological Society
R. G. E. Murray, *University of Western Ontario*
Canadian Society of Microbiologists
H. G. Thode, *McMaster University*
Chemical Institute of Canada
T. Thorvaldson, *University of Saskatchewan*, Royal Society of Canada

Ex officio

Léo Marion (Editor-in-Chief), *National Research Council*
F. T. Rosser, Director, Division of Administration, *National Research Council*

Manuscripts for publication should be submitted to Dr. Léo Marion, Editor-in-Chief, Canadian Journal of Chemistry, National Research Council, Ottawa 2, Canada.
(For instructions on preparation of copy, see **Notes to Contributors** (inside back cover).)

Proof, correspondence concerning proof, and orders for reprints should be sent to the Manager, Editorial Office (Research Journals), Division of Administration, National Research Council, Ottawa 2, Canada.

Subscriptions, renewals, requests for single or back numbers, and all remittances should be sent to Division of Administration, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and prices are:

Canadian Journal of Biochemistry and Physiology	Monthly	$3.00 a year
Canadian Journal of Botany	Bimonthly	$4.00 a year
Canadian Journal of Chemistry	Monthly	$5.00 a year
Canadian Journal of Microbiology	Bimonthly	$3.00 a year
Canadian Journal of Physics	Monthly	$4.00 a year
Canadian Journal of Technology	Bimonthly	$3.00 a year
Canadian Journal of Zoology	Bimonthly	$3.00 a year

The price of single numbers of all journals is 75 cents.

Canadian Journal of Chemistry

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 35 APRIL 1957 NUMBER 4

KINETICS OF THE THERMAL DECOMPOSITION OF HYDROGEN PEROXIDE VAPOR[1]

PAUL A. GIGUÈRE AND I. D. LIU

ABSTRACT

The rates of thermal decomposition of hydrogen peroxide vapor were measured by the static method at low pressures (0.2 to 20 mm. Hg), over the temperature range 300°–600° C., in carefully cleaned glass vessels. The reaction was of the first order with respect to time and the final products were only water and oxygen. Around 400° C. the character of the reaction changed gradually from heterogeneous (surface effects, low activation energy) to homogeneous (reproducible rates in various vessels). With initial pressures of about 10 mm. Hg the experimental rates above 400° lead to an apparent activation energy of 43 kcal. and a frequency factor of $10^{10.7}$. After correction for the residual surface decomposition, the rate equation becomes

$$k = 10^{13} \exp(-48{,}000/RT) \text{ sec.}^{-1}$$

in good agreement with the accepted value for the O—O bond dissociation energy. The reaction rates increased regularly with pressure.

Packing the reaction vessels with glass rods and adding various gases (including nitric oxide and propylene) had no appreciable effect on the gas-phase reaction. Deuterium peroxide vapor decomposed at the same rate as hydrogen peroxide under comparable conditions. The results may be explained adequately by the following non-chain mechanism for the uncatalyzed decomposition:

$$H_2O_2 = 2OH$$
$$OH + H_2O_2 = H_2O + HO_2$$
$$HO_2 + HO_2 = H_2O_2 + O_2$$
$$HO_2 + OH = H_2O + O_2$$

INTRODUCTION

One of the very first chemical reactions of which the rate was measured was the spontaneous decomposition of hydrogen peroxide (Thenard, 1818). Yet, in spite of countless investigations, our understanding of this deceptively simple reaction is still far from adequate. Obviously the major difficulty here comes from the extreme sensitivity of hydrogen peroxide to all sorts of catalysts. In recent years two independent investigators (15, 24) have shown that under suitable conditions, such as in a flow system, the homogeneous decomposition becomes measurable at temperatures somewhat above 400° C. Because of the uncertainty in analyzing the data in a flow experiment we felt that a study by the static method, if successful, would yield more reliable results, particularly for the activation energy. In fact we were able to follow the reaction at temperatures appreciably higher than heretofore (up to 600° C.) and it was possible to evaluate with good accuracy the relative importance of the surface and volume reactions.

EXPERIMENTAL

The reaction vessels, generally 2-liter flasks, were either of Pyrex or Vycor. First they were cleaned thoroughly with hot fuming sulphuric acid and, after they were rinsed,

[1]*Manuscript received September 24, 1956.*
Contribution from the Department of Chemistry, Laval University, Quebec, Que. This work was supported by the Office of Scientific Research of the United States Air Force under contract No. AF 18 (600)-492.

they were either fused in a blast lamp as described before (8), or treated for a few hours with hot, concentrated hydrogen peroxide. Even after such treatments the Pyrex flasks required some preliminary runs (a dozen or so) before yielding reproducible results. Vycor flasks, on the contrary, gave immediately consistent results. In addition they could be used safely above 500° C.

The reaction vessels were heated in a heavily insulated electric furnace (Fig. 1) the temperature of which was controlled to within ±0.3° and read to ±0.7° by means of

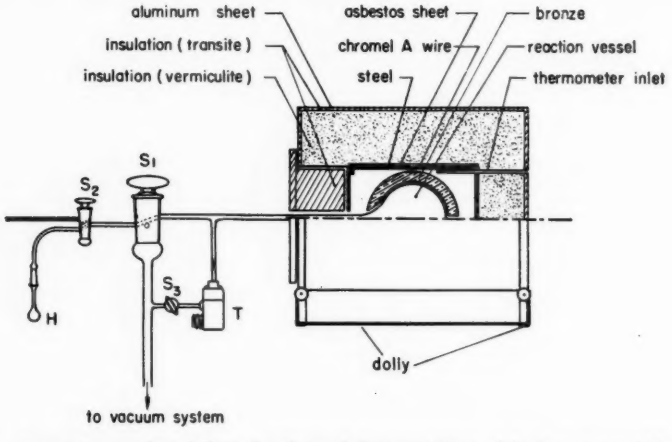


FIG. 1. Apparatus used to follow the thermal decomposition of hydrogen peroxide vapor.

an electronic thermometer. The sensitive element of the latter (platinum coil on quartz) was calibrated against a certified platinum resistance thermometer (Leeds and Northrup) up to 470° C. Beyond that range a large-scale, well adjusted extrapolation gave an accuracy estimated at 1.5° or better. Temperature gradients inside the furnace were minimized by enclosing the glass flasks within thick hemispheres of aluminum bronze. Aluminum foil provided good thermal contact between the flask and the metal shell. The thermometer bulb was inserted directly in a slot in one of the hemispheres.

A large three-way stopcock S_1, lubricated with silicone grease, connected the reaction chamber either with the vacuum system or with the reagents. The rate of the reaction was followed by recording the pressure increase with a pressure transducer T. The characteristics of these devices and their use in the present investigation have been described elsewhere (10). At first glass spoon-gauges coupled to a linear transducer were used; later this fragile combination was replaced by the all-metal transducers with identical results. The dead volume of the reaction system outside the furnace was only of the order of 50 cc.; these parts were kept at 90° C. to prevent condensation of the vapor. Tests confirmed that the decomposition of the peroxide vapor in the dead space was negligible. The reaction vessel was filled by evaporation of the sample of liquid peroxide kept in a small bulb H at 70°–80° C. Because of the low volatility of hydrogen peroxide the maximum pressure obtainable in a reasonable time by that technique was not higher than 20 mm. Hg. Higher pressures require boilers of special construction (21).

To study the effect of various added gases on the reaction rate an auxiliary reservoir was needed for mixing the reagents. To that end a 5-liter flask, kept at about 150° C.

in a heating mantle, was connected to the reaction system. The peroxide vapor was first introduced, as described above, then the other gas at a given partial pressure, from a storage vessel fitted with a second pressure transducer. The peroxide vapor mixed with the various gases used did not react measurably at 150° in the storage vessel. The preparation of the hydrogen peroxide samples 99.9% pure from Becco's 90% product has been described before. The deuterium peroxide, about 95%, was obtained by fractional distillation of the product from dissociation of heavy water in the electrical discharge (11). The various gases, presumably 98–99% pure, were taken directly from cylinders without further purification. Good reproducibility was achieved only by following rigorously the same experimental procedure. For instance, the reaction chamber had to be evacuated for the same length of time between runs (20 to 30 minutes depending on the temperature) and the initial pressure of reactants had to be constant in any given series. The experiments were scattered with respect to temperature to minimize any aging of the glass surface, albeit no sign of this ever appeared.

RESULTS

The majority of the some 700 runs carried out were in the temperature range 400°–500° C. and at initial pressures of 8 to 10 mm. Hg where the reproducibility was generally good (Fig. 2). As a rule consecutive runs yielded the same rate constant within the accuracy of measurements; non-consecutive runs with a given vessel generally agreed within 10 or 15%. All the runs (save a few in coated vessels) gave time–pressure curves strictly of the first order. Little attention was paid to the heterogeneous decomposition in the region below 400° C. as it has been studied extensively before (for a review cf. (22)). Its sole importance in this investigation was to provide a basis for correcting the rates at higher temperatures for any residual surface reaction. The apparent activation energy for this process was rather low, 10–12 kcal. as found before. The rates, however, were at least 30 times slower (for instance $k = 3\times10^{-4}$ sec.$^{-1}$ at 350° C.) than the slowest ones obtained previously under comparable conditions (8). As a consequence the transition temperature from heterogeneous to homogeneous decomposition was lowered to about 400° (Fig. 3).

In one series of runs the surface was increased by packing the reaction vessel with Pyrex tubes 8 mm. in diameter sealed at both ends under reduced pressure, and arranged as evenly as possible in space inside the flask. Increase in the surface-to-volume ratio from 0.4 to 0.7 and 1.1 cm.$^{-1}$ produced only a slight increase in the reaction rate above 400–425° C. but a more substantial rate increase of the heterogeneous reaction (Fig. 3). Likewise addition of various gases (air, oxygen, argon, and helium) had only minor effects on the rate as may be judged from Table I. In general an increase in total pressure accelerated the reaction slightly, the various gases being comparable in that respect. The curves of Fig. 4 illustrate the dependence of the reaction rate on the total initial pressure. The levelling off at low pressures is undoubtedly the result of increasing importance of the surface reaction. This is confirmed by the accompanying drop in apparent activation energy, down to 38 kcal. at 0.3 mm. Hg. The low-pressure limit at which the decomposition could be followed with the type of transducer used (Statham Laboratories, Model P97–0.05*d*) was of the order of 0.2 mm. Hg. The reaction was then slowed down enough to enable measurements up to 600° C. ($k = 2.5\times10^{-2}$ sec.$^{-1}$) in a 2-liter Vycor vessel.

Nitric oxide and propylene, commonly used as a test for free radicals in organic re-

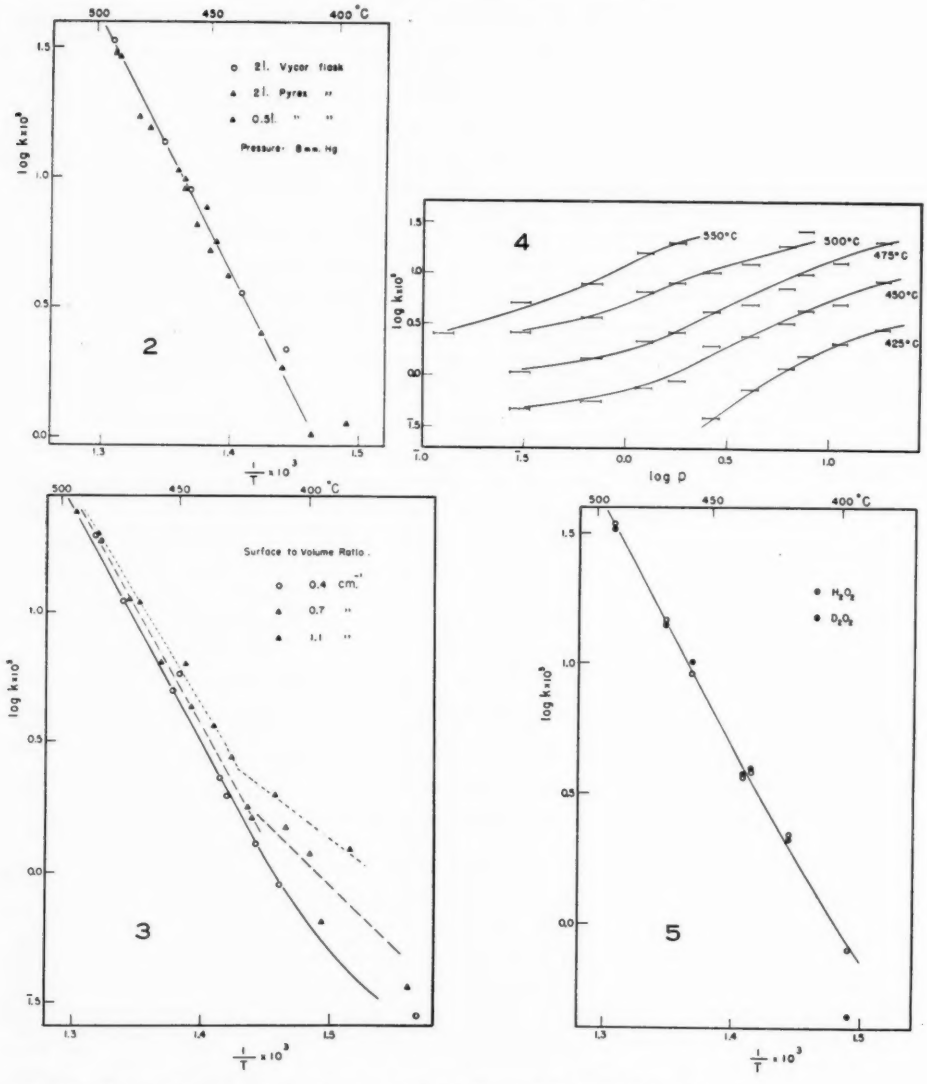


FIG. 2. Rates of decomposition of hydrogen peroxide vapor in different vessels.
FIG. 3. Effect of packing the vessels on the rate of decomposition of hydrogen peroxide vapor.
FIG. 4. Variation with pressure of the rate constant for the decomposition of hydrogen peroxide vapor.
FIG. 5. Compared rate constants for the vapor phase decomposition of hydrogen and deuterium peroxide.

actions, had no more effect on the reaction than the other gases reported above, at least in low concentrations (below 3%). Higher concentrations resulted in side reactions which were not investigated further. Hydrogen, in suitable quantities, led to mild explosions (second limit) but only after nearly all the hydrogen peroxide had decomposed. This interesting phenomenon is being studied in detail and will be described elsewhere. Under

TABLE I

EFFECT OF ADDED GASES ON THE RATE OF DECOMPOSITION OF HYDROGEN PEROXIDE VAPOR

Temp., °C.	Added gas	p_0, mm.Hg	$p_{(\text{added gas})}/p_0$	$t_{\frac{1}{2}}$, sec.
440	—	7	0	5.8
"	Air	7	0.13	5.8
"	"	7	0.50	5.8
"	"	11	0.32	7.1
"	Hydrogen	8	0.50	5.9
443	—	7	0	6.6
"	Argon	8	0.13	6.9
"	"	13	0.54	7.1
448	—	8	0	7.3
"	Oxygen	8	0.14	7.3
"	"	12	0.45	7.7
451	—	12	0	11
"	Air	12	0.1	11
"	Hydrogen	12	0.09	11
"	Helium	12	0.41	11
445	—	5	0	198
"	Propylene	5	0.05	174
467	—	7	0	78
"	Propylene	7	0.05	78
"	"	7	0.18	72
425	—	7	0	130
"	Nitric oxide	7	0.02	128
447	—	7	0	76
"	Nitric oxide	7	0.04	70
475	—	5	0	33
"	Nitric oxide	5	0.03	35
"	—	7	0	23
"	Nitric oxide	7	0.04	18

otherwise comparable conditions the vapor from more dilute hydrogen peroxide (90%) as well as that from the isotopic peroxide D_2O_2 (95%) was found to decompose at the same rate as the 99.9% material generally used (Fig. 5).

Coatings of fused salts were also tried as they have been claimed to be much more inactive than glass or quartz towards the decomposition of hydrogen peroxide (15). However, they were found unsuitable for measurements in a static system. Fused boric acid turned out to be very hygroscopic, the pressure of peroxide (or water) vapor decreasing quickly with time instead of increasing. Borax, even after it was fired at about 750° C., produced very fast, and approximately zero-order decomposition, resulting, no doubt, from diffusion to and from some active spots.

DISCUSSION

The Mechanism

The above results may be explained satisfactorily, if not uniquely, in terms of the following mechanism for the homogeneous decomposition:

$$H_2O_2 = 2OH \quad [1]$$
$$OH + H_2O_2 = H_2O + HO_2 \quad [2]$$
$$HO_2 + HO_2 = H_2O_2 + O_2 \quad [3]$$
$$HO_2 + OH = H_2O + O_2 \quad [4]$$

This sequence of elementary reactions involving no chains adds up to the over-all process

$$2H_2O_2 = 2H_2O + O_2. \quad [5]$$

We intend to show hereafter that other possible steps are either ruled out or less probable than the above for various reasons. Obviously, mere observations of pressure changes in the system are not sufficient for that purpose and we must rely strongly on evidence from other sources such as spectroscopy, mass spectrometry, electrical discharge work, etc.

It is now well established that the first act in the homogeneous decomposition of hydrogen peroxide is the fission of the O—O bond [1] following activation, thermal or otherwise. Ample evidence for this is provided by the high concentrations of OH radicals found spectroscopically in hydrogen peroxide vapor dissociated by ultraviolet light (28), in the flash photolysis (16) or in an electrical discharge (7). Recent attempts at pyrolysis with the toluene carrier technique have yielded sizable quantities of phenol (23). It must be mentioned, however, that in the *catalyzed* decomposition, no *free* hydroxyls seem to be present. This is indicated by the recent demonstration of Stone and Taylor (25) that carbon monoxide does not react with hydrogen peroxide vapor decomposing heterogeneously at about 110° C., whereas upon irradiation with actinic light the reaction

$$OH + CO = CO_2 + H \qquad [6]$$

occurs immediately.

The other conceivable initial step

$$H_2O_2 = HO_2 + H \qquad [7]$$

may be excluded on the grounds that (*a*) the O—H bond is twice as strong as the O—O bond, and (*b*) no molecular hydrogen is produced in the reaction. Indeed, it has been proved that both the catalyzed (22) and the photochemical decompositions (29) yield only water and oxygen after equation [5]. As a check we had the non-condensable gas, after complete thermal decomposition, analyzed by mass spectrometry. A sample from hydrogen peroxide decomposition at 470° C. showed 0.1% hydrogen, very near to the limit of detection of that method (0.08%), whereas a sample from deuterium peroxide showed no trace whatsoever of deuterium gas. Therefore, formation of free hydrogen atoms at any stage of the reaction is extremely improbable. Otherwise measurable amounts of hydrogen would appear in the final products as the results of secondary reactions such as:

$$H + H_2O = H_2 + OH \qquad [8]$$

and even

$$H + H + M = H_2 + M. \qquad [9]$$

Incidentally, the formation of free oxygen atoms in the homogeneous decomposition seems just as unlikely as that of hydrogen atoms. The reaction

$$H_2O_2 = H_2O + O \qquad [10]$$

sometimes postulated is certainly too complex for a single act in the gas phase. Similarly the reaction

$$2OH = H_2O + O \qquad [11]$$

suggested by Weiss (30) involves, like reaction [10], a forbidden transition from a singlet to a triplet electronic state. Accordingly, no O^+ ions have been found in the electron impact dissociation of hydrogen peroxide in a mass spectrometer although the conditions for their detection were very favorable (18).

The most probable step following the initial one is the attack of the parent compound by OH radicals [2], which must be extremely fast. This was confirmed recently by the mass

spectrometric investigations of Foner and Hudson (6) where the highest concentrations of HO_2 radicals were achieved by reacting OH radicals—from dissociated water or hydrogen peroxide vapor—with hydrogen peroxide at low pressures. Also in agreement is the spectroscopic observation that OH radicals disappear much more rapidly in dissociated peroxide vapor than in water vapor (7). Recombination of hydroxyl radicals

$$2OH + M = H_2O_2 + M \qquad [12]$$

need not be considered here since in a non-chain mechanism it results in no over-all reaction. At any rate the probability of such three-body collisions in the gas phase is negligible considering the very low steady-state concentration of OH radicals in the presence of hydrogen peroxide [2].

As for the chain-propagating step

$$HO_2 + H_2O_2 = H_2O + O_2 + OH \qquad [13]$$

first proposed by Haber and Willstatter (12), it must have an appreciable activation energy, because of its complexity, making it less probable than various termination steps in the gas-phase reaction. As shown below, the present results, as well as those for the photochemical decomposition (29), do not require a chain mechanism. Rather they disprove any appreciable contribution from chains. True, under certain conditions the decomposition of hydrogen peroxide becomes explosive. However, Satterfield and his co-workers (20, 21) have shown that these explosions must be of the thermal type since they usually require a high local temperature: ignition by a spark, by a heated filament, or by fast catalytic decomposition on some very active spot. From extrapolation of their data it would appear that in the high-pressure range of our measurements we were operating within the ignition limits of hydrogen peroxide vapor. Finally it may be recalled here that in the much studied reaction between hydrogen and oxygen, HO_2 radicals are always considered as leading to chain termination rather than propagation (14).

Of the various conceivable termination steps, the most likely are certainly the dismutation reactions [3] and [4]. Again the latter appears by far the less probable of the two, at least until the last stages of the decomposition, owing to the exceedingly strong competition of step [2] for OH radicals.

The Rate Equation

Because of its relatively high activation energy the first step [1] must be rate-determining. Consequently, if the decomposition were a truly elementary process its activation energy should be approximately equal to the bond dissociation energy D(HO—OH), on the very plausible assumption that the reverse reaction requires no activation energy. So far this quantity was taken as 51 kcal. (9) from pertinent thermochemical data and using the generally accepted value D(O—H) = 100.2 kcal. based on the equilibrium measurements of Dwyer and Oldenberg (4). However, this numerical value was admittedly not beyond doubt and it was hoped that the measurements could be repeated with more accuracy (17). Later on they were shown to be open to some criticism (5); an assumed linear relationship between absorption intensity and concentration of OH would tend to make the above value a lower limit. Very recently Barrow and Downie (1) have reported an accurate spectroscopic determination involving only a short Birge-Sponer extrapolation and leading to a slightly higher value of D(O—H), $101.4_8 \pm 0.5$ kcal. This is considered the most reliable to date (13) and we will adopt it for the present

discussion. Consequently the value of D(HO—OH) is found to be 48.5 kcal. from accepted heats of reaction reduced to 0° K. (9, 19).

Now the present reaction rates measured at initial pressures of about 10 mm. Hg and at temperatures above 400° C. may be fitted fairly closely to the equation

$$k = 10^{10.7} \exp(-42{,}500/RT) \text{ sec.}^{-1}$$

A discrepancy of 6 kcal. is certainly beyond the estimated uncertainty of our experimental activation energy. On further analysis of the results, however, we reflected that the observed rate constants, k_e, were the sum of two terms: k_g for the gas-phase reaction and k_s for the residual surface reaction. The experimental rates should, therefore, be corrected for the latter, a fairly important correction particularly in the transition range, 400°–430° C. To that end the low-temperature portion of the Arrhenius curve extending down to about 300° (Fig. 3) was extrapolated as accurately as possible into the region of homogeneous decomposition and the approximate k_s thus obtained were subtracted from the measured k_e above 400° C. Because of the mutual interference of the two

TABLE II

REACTION RATE CONSTANTS CORRECTED FOR THE RESIDUAL SURFACE DECOMPOSITION IN 2-LITER FLASKS

Temp., °C.	k_e	k_g	k_g/k_e
	A. Acid treated Pyrex flask		
396	1.07	0.661	61.8
431	6.22	5.73	92.1
437	6.46	5.84	90.4
450	11.1	10.4	93.7
454	11.6	10.9	94.0
458	9.82	9.06	92.3
470	23.1	22.3	96.5
471	29.6	28.7	96.9
491	57.8	56.8	98.3
Average E_{act}	44.5 kcal.	48.1 kcal.	
	B. Flask A after fusing		
401	1.11	0.681	61.3
402	1.50	1.06	70.7
411	5.78	5.30	91.7
428	4.34	3.77	86.9
430	3.99	3.41	85.5
433	4.52	3.92	86.7
456	13.0	12.3	94.6
456	13.8	13.1	94.9
467	20.0	19.2	96.0
471	26.4	25.5	96.7
482	46.2	45.3	98.1
488	53.3	52.3	98.1
Average E_{act}	44.5 kcal.	48.1 kcal.	
	C. Peroxide treated Vycor flask		
384	0.315		
397	0.520	1.107	20.6
410	1.61	1.14	70.8
412	0.82	0.334	40.7
429	3.74	3.16	84.5
442	5.72	5.04	89.1
449	7.85	7.15	91.1
455	10.0	9.26	92.6
460	11.0	10.2	92.7
480	25.3	24.1	95.3
Average E_{act}	43.0 kcal.	48.4 kcal.	

decomposition processes this correction yields only a first approximation. However, no significant improvement was obtained after iteration. The recalculated rate constants for three different reaction vessels are given in Table II, from which it is seen that the average activation energy for the homogeneous process alone, $E = 48 \pm 3$ kcal., agrees well with the above value of D(HO—OH). The graph in Fig. 6 shows that in our experi-

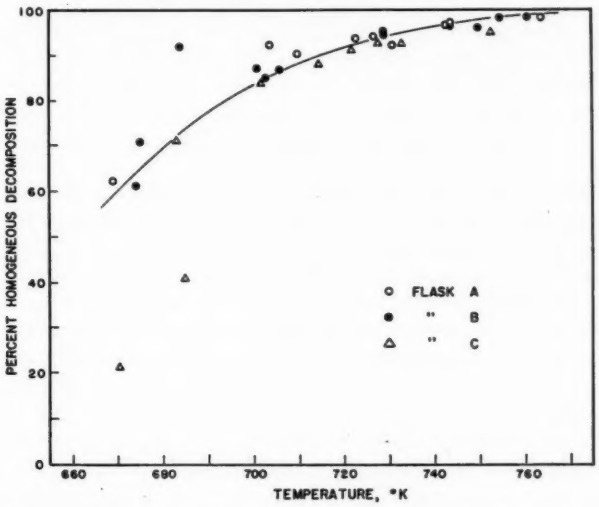


FIG. 6. Fraction of hydrogen peroxide vapor decomposing homogeneously at various temperatures in 2-liter flasks.

ments the decomposition was homogeneous to the extent of about 60% at 400° C. and 90% at 450° C. in 2-liter vessels. Obviously the uncertainty here is greater than for the original data. The results for the decomposition of deuterium peroxide were not numerous enough for similar calculations; at any rate expected difference between the dissociation energy of the two isotopic molecules (about 1 kcal.) is well within the accuracy of our measurements.

In the above reasoning a number of minor points have been overlooked, such as the following two, for instance: (*a*) The transition state theory of unimolecular reactions (26) predicts that the observed activation energy will be greater than the corresponding bond energy by RT, i.e. some 1.5 kcal. in our case. (*b*) The decomposition of gaseous hydrogen peroxide is exothermic by about 26 kcal. at 400°–500° C. Now, assuming that convection is unimportant, the mean time for establishment of stationary states in peroxide vapor is found to be of the order of half a minute in a 500 ml. flask and 1 minute in a 2-liter flask. Therefore, the self-heating effect becomes appreciable in the higher temperature and pressure ranges, as may be judged from the figures in Table III calculated by the method of Benson (3). This effect, like the previous one, is still within the estimated accuracy of the calculated activation energy.

Substitution of the corrected activation energy 48 kcal. in the Arrhenius equation leads to a frequency factor A near the expected value for a unimolecular reaction 10^{13}.

We are inclined to consider the excellent agreement of our recalculated results with the predictions of the theory as a confirmation that the homogeneous decomposition of

TABLE III

TEMPERATURE GRADIENTS IN THE DECOMPOSITION OF HYDROGEN PEROXIDE VAPOR

	Initial pressure, mm. Hg					
	5		10		15	
	Temperature, ° C.					
Size of vessel	430°	480°	430°	480°	430°	480°
500 ml.	0.25°	1.4°	0.68°	4.3°	1.1°	6.6°
2 liters	0.64°	3.6°	1.7°	11°	2.7°	17°

hydrogen peroxide vapor proceeds mainly, if not exclusively, through initial splitting into OH radicals followed by fast, non-chain reactions. Any appreciable contribution from chains would imply higher rates than those observed. In point of fact this constitutes stronger evidence against a chain mechanism than the few tests mentioned above. Thus the negative effect from a threefold increase of the surface-to-volume ratio (Fig. 3) could be explained if chains were initiated *and* broken on the surface. Likewise, the failure of such standard chain inhibitors as nitric oxide and propylene (Table I) is of no definite significance here because so little is known of the reactions of these molecules with hydrogen peroxide.

According to the theory of simple unimolecular reactions (27) the specific reaction rate at a given temperature approaches a constant value k_∞ at a certain limiting value of the concentration or pressure, as shown in Fig. 5. (As explained before these curves do not fall off steadily at lower pressures because of increasing relative importance of the surface reaction.) Now a rough estimate of this upper-limit pressure may be made from the relationship derived by Benson (2) between the average rate of decomposition $\bar{k}(\epsilon)$ and the normal vibration frequencies of the activated complex ($\bar{\nu}$). Using for the latter some plausible averages, around 1000 cm.$^{-1}$ as in most bond-breaking reactions, we find, for the present case, $\bar{k}(\epsilon) = 3.1\times10^{8}$ sec.$^{-1}$ at 475° C. The collision frequency z^0 at that temperature being 3×10^{-10} sec.$^{-1}$ and the number of molecules per unit volume $N^0 = 1.3\times10^{16}\times p$, the limiting pressure is found to be

$$p = 3\times10^{8}/1.3\times10^{16}\times3.1\times10^{-10} = 75 \text{ mm. Hg.}$$

A short extrapolation of the curves in Fig. 4 confirms that this result is roughly of the right order of magnitude. Because of the experimental difficulties involved we have not yet succeeded in checking that point.

In conclusion the above results emphasize the futility of attempting to measure the purely homogeneous decomposition of hydrogen peroxide at ordinary temperature. From the rate equation for the thermal reaction it is clear that in the complete absence of catalysts and high-energy radiation hydrogen peroxide would be indefinitely stable at moderate temperatures (average life of the order of 10^7 years at 100° C.).

RÉSUMÉ

On a mesuré la vitesse de décomposition du peroxyde d'hydrogène à l'état gazeux dans un système statique sous des pressions allant de 0.2 à 20 mm. de mercure et dans l'intervalle de température entre 300° et 600° C. La réaction était toujours du premier ordre et les seuls produits étaient la vapeur d'eau et l'oxygène. Au-dessus de 400° C. et

dans des récipients de verre soigneusement nettoyés la réaction homogène l'emportait sur la réaction de surface, et l'énergie d'activation passait brusquement de 10 ou 12 kcal. à environ 43 kcal. En corrigeant les résultats expérimentaux pour la décomposition catalytique on arrive à l'expression

$$k = 10^{13} \exp(-48000/RT) \text{ sec.}^{-1}$$

pour la réaction homogène entre 400° et 500° C. et sous des pressions supérieures à 10 mm. L'énergie d'activation ainsi obtenue est à peu près égale à l'énergie de dissociation de la liaison O—O. Le peroxyde de deutérium se décompose à la même vitesse que le peroxyde d'hydrogène dans les mêmes conditions. Les résultats peuvent s'expliquer adéquatement par une suite de réactions élémentaires, sans chaînes.

ACKNOWLEDGMENTS

The authors are grateful to their colleagues Prof. C. Ouellet and Dr. P. Ausloos for many helpful discussions, and to Dr. F. Lossing and Miss F. Gauthier from the National Research Council, Ottawa, for the mass spectrometric analyses.

REFERENCES

1. Barrow, R. F. and Downie, A. R. Proc. Phys. Soc. A, **69**, 178 (1956).
2. Benson, S. W. J. Chem. Phys. **20**, 1064 (1952).
3. Benson, S. W. J. Chem. Phys. **22**, 46 (1954).
4. Dwyer, R. J. and Oldenberg, O. J. Chem. Phys. **12**, 351 (1944).
5. Edse, R. Third Symposium on Combustion Flame and Explosion Phenomena. Williams & Wilkins Co., Baltimore. 1949. Paper No. 79, p. 611.
6. Foner, S. N. and Hudson, R. L. J. Chem. Phys. **23**, 1364 (1955).
7. Frost, A. A. and Oldenberg, O. J. Chem. Phys. **4**, 781 (1935).
8. Giguère, P. A. Can. J. Research, B, **25**, 135 (1947).
9. Giguère, P. A. and Liu, I. D. J. Am. Chem. Soc. **77**, 6477 (1955).
10. Giguère, P. A. and Liu, I. D. Can. J. Technol. **34**, 473 (1957).
11. Giguère, P. A., Secco, E. A., and Eaton, R. S. Discussions Faraday Soc. **14**, 104 (1953).
12. Haber, F. and Willstatter, R. Ber. **64**, 2844 (1931).
13. Herzberg, G. Private communication.
14. Lewis, B. and von Elbe, G. Combustion, flames and explosions of gases. Academic Press, Inc., New York. 1951.
15. McLane, C. K. J. Chem. Phys. **17**, 379 (1949).
16. Norman, I. and Porter, G. Proc. Roy. Soc. A, **230**, 399 (1955).
17. Oldenberg, O. J. Chem. Phys. **17**, 1059 (1949).
18. Robertson, A. J. B. Trans. Faraday Soc. **48**, 228 (1952).
19. Rossini, F. D. Selected values of thermodynamic properties. Natl. Bur. Standards (U.S.) Circ. 500. 1952.
20. Satterfield, C. N., Ceccotti, P. J., and Feldbruge, A. H. R. Ind. Eng. Chem. **47**, 1040 (1955).
21. Satterfield, C. N., Kavanagh, G. M., and Resnick, H. Ind. Eng. Chem. **43**, 2507 (1951).
22. Schumb, W. C., Satterfield, C. N., and Wentworth, R. L. Hydrogen peroxide. Reinhold Publishing Corp., New York. 1955.
23. Sehon, A. Private communication.
24. Stein, T. W. Unpublished results. Quoted in Ref. **22**.
25. Stone, F. S. and Taylor, H. S. J. Chem. Phys. **20**, 1339 (1952).
26. Szwarc, M. Chem. Revs. **47**, 75 (1950).
27. Trotman-Dickenson, A. F. Gas kinetics. Butterworths, London. 1955.
28. Urey, H. C., Dawsey, L. H., and Rice, F. O. J. Am. Chem. Soc. **51**, 137 (1929).
29. Volman, D. H. J. Chem. Phys. **17**, 947 (1949).
30. Weiss, J. Trans. Faraday Soc. **36**, 856 (1940).

SURFACE STUDIES USING ION EXCHANGE AUTOCHROMATOGRAPHY[1]

RUTH E. KREHBIEL[2] AND J. W. T. SPINKS

ABSTRACT

In order to investigate surface exchange phenomena in detail, a new method, the method of ion exchange autochromatography, has been developed. Its application to the study of finely divided precipitates of strontium sulphate is described.

INTRODUCTION

The heterogeneous exchange between the ions on the surface of an ionic crystal and the ions in a solution has been widely used as the basis for a radioactive method for determining the "active" surface area of finely divided solids (for recent summaries see 7, 17). Tracers isotopic with either the cation or the anion of the crystals may be used and should, on the basis of simple theory, yield the same calculated surface area. This is indeed the case in experiments in which doubly labelled saturated $PbSO_4$ solutions are shaken with $PbSO_4$ precipitates, but is not the case when saturated solutions containing $Sr^{90}S^{35}O_4$ are shaken with $SrSO_4$ precipitates (9, 12). For the latter, the apparent areas, as determined using Sr^{90} ion and $S^{35}O_4^=$, are in the ratio of about 2: 1. If one wished to investigate examples of this behavior, in which however the differences were less marked, it would be possible to improve the procedure by shaking the same doubly labelled solution with successive fresh portions of precipitate. In this way small differences would be amplified as in successive batch extractions. Such successive batch extractions are often more conveniently carried out in a column, as in column chromatography.

Ion exchange chromatography is, of course, already a well established technique and has led to such accomplishments as the separation of rare earth elements by Spedding and his co-workers (10) and the development, on the theoretical side, of a theory of partition chromatography based on the equilibrium stage concept (4) which has since been applied to ion exchange chromatography (6, 16).

Typical ion exchange resins have yielded very high values for the number of theoretical plates per unit length of column (E. G. Dowex 50; 100 plates per cm. in Ref. 1).

Marked isotope effects have been observed for hydrogen (2), carbon (8), sodium (1), lithium (13), potassium (13), and nitrogen (11), the column experiments of Spedding, Powell, and Svec with nitrogen being particularly striking.

The application of the chromatographic method to the present problem leads to *ion-exchange autochromatography*, in which the solute in the eluant and the material of the column packing are identical. Thus in the present experiments a column of $SrSO_4$ precipitate is eluted with saturated doubly labelled $SrSO_4$ solution.

EXPERIMENTAL

Materials and Methods

Radioactive strontium.—Sr^{89} was obtained as strontium carbonate from the Atomic Energy of Canada Ltd.; activity, 0.41 mc. in 2.63 g. $SrCO_3$.

Radioactive sulphur.—S^{35} was obtained as carrier free sulphuric acid in dilute HCl solution from the Atomic Energy of Canada Ltd.; specific activity, 0.5 mc. per ml.

[1]*Manuscript received November 1, 1956.*
Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan.
[2]*Present address: National Research Council, Ottawa, Canada.*

Preparation of radioactive $Sr^{89}S^{35}O_4$ precipitate and saturated solution.—The 2.63 g. $Sr^{89}CO_3$ was dissolved in 50 ml. dilute hydrochloric acid solution. Two milliliters of this solution was added to a mixture of 200 λ active sulphuric acid solution in 5 ml. 0.18 *N* H_2SO_4 solution in a centrifuge tube. The tube was heated in a boiling water bath for 30 minutes. At the end of this time the mixture was centrifuged, the supernatant decanted, and the precipitate washed five times with water. The active $Sr^{89}S^{35}O_4$ precipitate was then transferred to a glass stoppered liter flask, filled with distilled water. The solution was shaken at intervals over a period of 3 days at room temperature (25° C.), after which time the specific activity of the solution remained constant and the solution was assumed to be saturated.

Preparation of inactive $SrSO_4$ precipitate.—Two hundred and ten grams of $Sr(NO_3)_2$ was dissolved in 750 ml. distilled water, heated to 60° C., and filtered. To this hot solution was added, with stirring, 120 ml. hot 9*M* H_2SO_4 solution and the resulting mixture was gently boiled for 20 minutes. Three portions of $SrSO_4$ precipitate, prepared in this way, were combined and washed with 10 successive 3 liter portions of distilled water. The precipitate was then transferred to a wide mouthed, glass stoppered bottle and allowed to age 1 week before use. In a shaking type exchange experiment, this precipitate showed the type of behavior described by Singleton and Stow (9, 12).

Description of column.—The column was made of Pyrex glass, 31 cm. long, with an inner diameter of 12 mm. Surrounding the column was a water jacket of outer diameter 22 mm. The bottom of the column was joined to a 24/40 ground-glass joint. A sintered glass disk of fine porosity was sealed into the upper end of a corresponding 24/40 ground-glass joint, the bottom of which was tapered to fit a hole in a rubber stopper. The joint facilitated both the extrusion of the material in the column and the cleaning of the column.

Measurement of radioactivity.—Activities of samples were measured using an end window type geiger tube, window thickness 3.5 mg./sq. cm., and scaler (Nuclear Instrument and Chemical Co. model 165), with the usual corrections being made for background. Standard aluminum counting dishes, 3.1 cm. diameter, were used in a fixed geometry. In each case, 2 ml. of the solution to be counted was pipetted into the dish and evaporated by means of a heat lamp. Since the solubility of $SrSO_4$ is only 0.110 g. per liter, a negligible weight of precipitate remains and self-absorption can be neglected. Each sample was counted twice, once to get the combined activity of Sr^{89} and S^{35} and again through a 21.2 mg./cm.^{2} aluminum absorber. This was found experimentally to stop all the sulphur radiation* but transmitted 82% of the strontium radiation, and it was then possible to calculate what the separate activities of both strontium and sulphur would be in the absence of the absorber.

General Experimental Procedure (3)

The column was fitted by means of a one-holed rubber stopper to a 500 ml. suction flask and clamped in an upright position. The wet precipitate was poured into the column and packed by means of suction applied to the flask. When the liquid head above the precipitate had just disappeared, the suction was discontinued and a suitable volume of saturated $SrSO_4$ solution, labelled in both the Sr^{++} and $SO_4^{=}$, was added at the top of the column and drawn through the column by suction. Following this, successive additions of 5 ml. portions of inactive saturated solution were made. The eluate was collected in 5 ml. portions in test tubes.

**The range for S^{35} is greater than 21 mg./cm.^{2} Part of the stopping is due to the counter window and a small thickness of air.*

Two milliliters of each eluate fraction was measured into a counting dish and evaporated for counting. The measured activities were plotted as the per cent of the activity in a 2 ml. sample of the original saturated active solution added to the column, versus the total volume eluted.

In some experiments the distribution of the activity on the column was determined. After the passage of the active solution through the column, the column material was extruded and cut into slices. The slices of precipitate were placed on counting dishes, and dried and counted. The measured activities were corrected for self-absorption using a correction curve. A self-absorption curve was determined by successively adding layers of active precipitate to counting dishes; each time the precipitate was dried, weighed, and both S and Sr were counted. The results were plotted as activity against precipitate weight. It was found that for sulphur the count remained constant for a precipitate weight of 0.4 g. or more in a dish 3.1 cm. in diameter. This meant that any sample over 0.4 g. in weight constituted an infinitely thick sample for counting sulphur activity. The strontium count rose only slowly relative to precipitate weight after about 2 g.

The self-absorption curve was then converted to a correction curve (Fig. 1) in which the activity at 2.70 g. precipitate weight was arbitrarily taken to be 100% and the other points were plotted relative to it. Activities of column slices were then corrected to the standard 2.70 g. weight.

Several experiments were also carried out at elevated temperatures. This was accomplished by pumping water from a constant temperature bath through the jacket surrounding the column, and proceeding with the rest of the experiment in the manner already described.

Results for a Typical Elution Experiment

In a typical elution experiment the column was packed with $SrSO_4$ precipitate to a height of 11 cm. Fifty milliliters of active saturated solution was then added to the column and drawn through by suction applied to the arm of the flask. The 50 ml. resulting eluate was discarded. The column was next eluted with 210 ml. inactive saturated $SrSO_4$ solution. The inactive solution was added 5 ml. at a time with a pipette. The eluate was collected in 5 ml. portions in test tubes placed in the suction flask, the pump being disconnected after each 5 ml. had passed through the column. The average flow rate

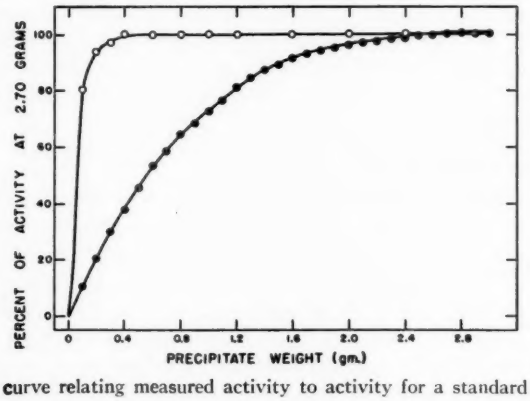
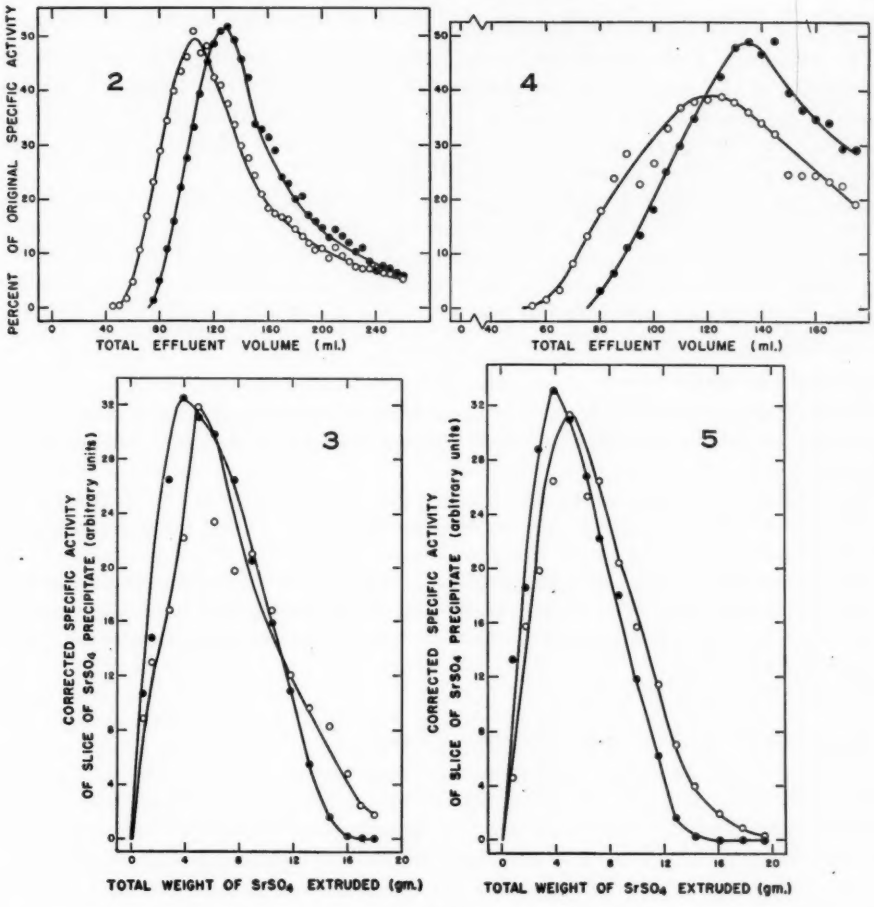


FIG. 1. Correction curve relating measured activity to activity for a standard precipitate weight of 2.7 g.; ●, Sr; ○, S.



FIGS. 2 and 4. Elution curve for strontium sulphate; sample activity as per cent of original specific activity versus total effluent volume (25° and 36° C. respectively): ●, Sr; ○, S.
FIGS. 3 and 5. Distribution of Sr^{89} and $S^{35}O_4^{=}$ on a column of precipitated strontium sulphate (25° and 36° C. respectively): ●, Sr; ○, S.

through the 11 cm. column was 0.56 ml./minute. The temperature of the column was 25° C. Two milliliters of the 5 ml. sample was pipetted into an aluminum counting dish and evaporated under a heat lamp. The dried samples were then counted both for total activity and Sr activity using the 21.2 mg./cm.^{2} absorber. Two milliliters of the original solution was also counted for S^{35} and Sr^{89} activity. The counting time was 10 minutes for each sample. The results are given in Fig. 2 in which the activities of the effluent samples are plotted as per cent of original specific activity versus total effluent volume.

The 11 cm. of precipitate was extruded from the column using a rubber disk, which fitted the inside of the column, and a glass rod. The wet precipitate was weighed, dried at 110° C., and weighed again. From this it was determined that the free volume in the column was 8.75 ml. and that the precipitate dry weight was 17.89 g.

Results for a Typical Extrusion Experiment

The conditions of the above experiment were closely approximated to determine the distribution of the activities on the column. Fifty milliliters active solution was added to an 11 cm. column of precipitate at 25° C. The flow rate through the column was 1 ml./minute. The column was then rapidly washed with 10 ml. distilled water to remove most of the activity not adsorbed on the column.

The column was then extruded, and the precipitate sliced, dried, weighed, and counted as described earlier. Using Fig. 1, the measured activity of each sample was corrected to the activity which would have been obtained if the sample weight had been 2.70 g. The sulphur counts were also multiplied by 6 to make them comparable to the strontium counts. The results are plotted in Fig. 3 as activity of the slice of precipitate versus cumulative weight of precipitate, zero weight indicating the top of the column.

Experiments at 36° C.

The ordinary elution experiment was also carried out at a somewhat higher temperature by the circulation of water from a constant temperature bath through the jacket surrounding the column.

The results of an experiment carried out at 36° C. are plotted in Fig. 4. In this case 50 ml. active solution and 130 ml. inactive solution were passed through the 11 cm. column at a flow rate of 0.63 ml./minute.

The free volume of the column was 8.10 ml. and the precipitate weight was 19.68 g.

The distribution of activity on the column was also determined at 36° C. Again 50 ml. active solution was passed through an 11 cm. column, and the column was washed with 10 ml. distilled water.

The results are plotted in Fig. 5.

Remarks Concerning Additional Experimental Results

A total of 22 elution experiments were carried out and all displayed the characteristic trend of the $S^{35}O_4^=$ appearing in the eluate before the Sr^{89} ion and the sulphur reaching its activity peak before the strontium. In the extrusion experiments the strontium activity was always concentrated closer to the top of the column than the sulphur activity.

DISCUSSION

The foregoing elution experiments confirm the earlier evidence of Singleton (9) and Stow (12) that the exchange properties of Sr^{++} and $SO_4^=$ ions in the surface of a $SrSO_4$ precipitate are different. The separation of $S^{35}O_4^=$ and Sr^{89} in a typical elution experiment was demonstrated conclusively.

In all elution experiments, the S activity appeared before the Sr activity in the eluate. Also, the Sr^{++} was adsorbed closer to the top of the column than was the $SO_4^=$ ion. These results are qualitatively in agreement with the amount of exchange as determined by Stow, Singleton, and Spinks, the strontium being adsorbed more strongly than the sulphur.

Attempts to analyze the problem quantitatively have not proved very successful. From the general similarity of the present experiments to column chromatography it is tempting to apply the theory developed for resin columns (6, 14, 15). However, an exact application of the theory is out of the question since, owing to the limited solubility of $SrSO_4$, 5.6 column volumes (50 ml.) were used to dissolve the radioactive sample whereas

a negligibly small sample should be used. Thus the quantitative values for the number of theoretical plates, based on this analogy, are not of much value (from Fig. 2, 11.9 plates for sulphur and 17.2 plates for strontium).

The equilibrium distribution coefficients between solutes and resin can ordinarily be used to calculate the positions of the elution peaks. In Fig. 2, the ratio of the peak volumes is 14.8/12.2 = 1.21, which is much smaller than the ratio of the distribution coefficients reported earlier (approximately 1.9 (12)). However, this discrepancy might well be due to the fact that the precipitates used were made at different times and might, therefore, have had somewhat different surface properties. The same explanation would also account for the fact that the peaks occur much earlier than one would have expected from the equilibrium values.

The elution curves are not completely symmetrical but show instead a considerable tail as compared to published curves for substances such as the rare earths adsorbed on resins (5). According to theory, the curves become the more symmetrical the closer the approach to equilibrium conditions, and it would thus seem that the present experiments were carried out under non-equilibrium conditions. The upper limit to the flow rate used was determined by the pressure which could be applied in our particular experiments. Most experiments were carried out near this upper limit as a matter of expediency since it enabled one to complete an experiment in the course of a day. Any considerable lowering of the flow rate, say by a factor of 10, would have lengthened the experiment unreasonably.

Actually, equilibrium experiments (9, 12) have shown that surface exchange is virtually complete within a few minutes so that the major cause of this asymmetry cannot reasonably be attributed to non-equilibrium conditions across the film of liquid adjacent to the crystal surface. It may, however, be due to the superimposed slower exchange involving ions within the bulk of the crystal (possibly in cracks or other surface defects). Previous equilibrium studies (see Fig. 1, Ref. 12) have indicated that under the present experimental conditions a very appreciable fraction (25% or more) of the radioactive ions may become involved in the latter type of exchange. Since this process is very slow, it could account qualitatively for the very pronounced "tail" in the elution curves. The anomalous effect of increase in temperature is in qualitative agreement with this; increasing the temperature increases the fraction of ions penetrating the bulk of the crystal and hence broadens the elution peaks as observed. (This is opposite to the usual effect where increasing the temperature causes the ion exchange column to operate closer to equilibrium and hence increases the number of theoretical plates (15).)

Complete separation of all the Sr^{89} and all the $S^{35}O_4^=$ was not obtained, although the first fraction of $S^{35}O_4^=$ coming through was free from Sr^{89}. This result is to be expected from the mathematical considerations of a column (5). There always will be some overlapping between the elution curves since the column must be infinitely long to secure complete separation. The degree of separation will be dependent on the difference in equilibrium distribution coefficients.

It would be desirable to do experiments, using high specific activity $SrSO_4$, which might be expected to parallel the resin column experiments more closely.

By using two separately measurable isotopes of the same element, isotope separation occurring in surface exchange phenomena could also be studied and might be expected to be of considerable theoretical (and possibly practical) importance. It would seem that situations parallel to the above experiments must occur quite often in nature and would lead to appreciable isotopic fractionation.

ACKNOWLEDGMENT

We are grateful to the National Research Council of Canada for financial assistance.

REFERENCES

1. BETTS, R. H., HARRIS, W. E., and STEVENSON, M. D. Can. J. Chem. **34**, 65 (1956).
2. BROWN, W. G., KAPLAN, L., VAN KYKEN, A. R., and WILZBACH, K. E. Intern. Conf. Peaceful Uses of Atomic Energy. A/conf. 8/P/149 U.S.A. 28 June, 1955.
3. KREHBIEL, R. E. M.A. Thesis, University of Saskatchewan, Saskatoon, Sask. 1956.
4. MARTIN, A. J. and SYNGE, R. L. M. Biochem. J. **35**, 1385 (1941).
5. MAYER, S. W. and TOMPKINS, E. R. J. Am. Chem. Soc. **69**, 2866 (1947).
6. NACHOD, F. C. Ion exchange theory and application. Academic Press, Inc., New York. 1949.
7. PANETH, F. A. Radio-elements as indicators. McGraw-Hill Book Company, Inc., New York. 1928.
8. PIEZ, K. A. and EAGLE, H. Science, **22**, 968 (1955).
9. SINGLETON, R. H. and SPINKS, J. W. T. Can. J. Research, B, **27**, 238 (1949).
10. SPEDDING, F. H. and POWELL, J. E. J. Am. Chem. Soc. **76**, 2545 (1954).
11. SPEDDING, F. H., POWELL, J. E., and SVEC, H. J. J. Am. Chem. Soc. **77**, 1939 (1955).
12. STOW, R. M. and SPINKS, J. W. T. Can. J. Chem. **33**, 938 (1955).
13. TAYLOR, T. I. and UREY, H. C. J. Chem. Phys. **6**, 429 (1938).
14. TOMPKINS, E. R. J. Chem. Educ. **26**, 32 (1949).
15. TOMPKINS, E. R. J. Chem. Educ. **26**, 92 (1949).
16. TOMPKINS, E. R. and MAYER, S. W. J. Am. Chem. Soc. **69**, 2859 (1947).
17. WAHL, A. C. and BONNER, N. A. Radioactivity applied to chemistry. John Wiley & Sons, Inc., New York. 1951. p. 34.

THE OLEFINIC BOND IN GELSEMINE[1]

LÉO MARION AND K. SARGEANT[2]

ABSTRACT

The readily reducible double bond of gelsemine has been shown to be present in a vinyl side chain and not in an exocyclic methylene group as had been previously assumed. Oxidation of the olefinic double bond in N(a)-methylgelsemine with sodium metaperiodate in the presence of a catalytic amount of osmium tetroxide gave an aldehyde having one carbon less than the starting material. That the product was an aldehyde and not a ketone was proved (*a*) by conversion to an oxime which was readily dehydrated to a nitrile, and (*b*) by Wolff-Kishner reduction to a compound containing a C—CH_3 group not present in the aldehyde itself.

The presence in gelsemine of one readily reducible double bond was first reported by Chu and Chou (1), who prepared dihydrogelsemine. Gibson and Robinson (2) have shown by the Kuhn-Roth method that dihydrogelsemine contains a C—CH_3 group, whereas gelsemine contains no such group. They concluded that an exocyclic methylene group was present in the alkaloid. Later Goutarel, Janot, Prelog, Sneeden, and Taylor (3) obtained formaldehyde on ozonolysis of gelsemine and interpreted this as confirming the conclusion of Gibson and Robinson. The exocyclic methylene group has since been generally accepted.

At a meeting of the American Chemical Society, Dr. E. Wenkert first questioned the validity of the conclusion drawn by Gibson and Robinson (2) and mentioned observations in his laboratory indicating that the olefinic double bond of gelsemine could well be present in a vinyl group. This prompted a re-examination of the problem.

The infrared absorption spectrum of gelsemine contained two absorption peaks, one at 991 $cm.^{-1}$ and the other at 909 $cm.^{-1}$, that could be attributed to a vinyl group. The spectrum of dihydrogelsemine no longer contained a band at 909 $cm.^{-1}$ but showed a sharp peak at 996 $cm.^{-1}$. It is therefore difficult to decide unequivocally the nature of the substitution about the double bond from the infrared evidence, since only one of the two bands seems to disappear on removal of the olefinic bond. On the other hand, the presence in dihydrogelsemine of a C—CH_3 which is absent in gelsemine (2) and the formation of formaldehyde in the ozonolysis of the alkaloid (3) are both compatible with either an exocyclic methylene group or a vinyl side chain. We now wish to place on record experiments which afford a rigorous proof of the presence of a vinyl group in gelsemine.

The nuclear magnetic resonance spectrum of dihydrogelsemine showed a triplet peak which was absent in that of gelsemine. Were an exocyclic methyl group present in the former compound instead of an ethyl group, a doublet would have been observed. Furthermore, the oxidation of dihydrogelsemine with chromic acid according to the method of Lemieux and Purves (5) gave rise to a mixture of acetic and propionic acids.[3] The production of propionic acid indicates the presence of an ethyl group in dihydrogelsemine.

In view of these preliminary indications of the presence of a vinyl side chain in the

[1]*Manuscript received December 6, 1956.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 4232.

[2]*Postdoctorate Fellow of the National Research Council of Canada, 1954–1956.*

[3]*This oxidation, separation of the acids by chromatography on Celite, and characterization of the acids by the infrared spectra of their sodium salts in potassium bromide pellets was kindly carried out by Dr. R. U. Lemieux and Mr. J. P. Barrette at the University of Ottawa.*

alkaloid, an attempt was made to isolate the basic residue remaining after scission of the olefinic linkage.[4] Use was made of a recently introduced simplification of the older method of oxidation with osmium tetroxide. It has been shown by Pappo, Allen, Lemieux, and Johnson (6) that sodium metaperiodate, in the presence of catalytic amounts of osmium tetroxide, may be used to cleave olefinic linkages under mild conditions. Application of the method of oxidation to N(a)-methylgelsemine ($C_{21}H_{24}O_2N_2$) gave in good yield a new compound ($C_{20}H_{22}O_3N_2$) having lost CH_2 and containing one more oxygen. The infrared absorption spectrum of N(a)-methylgelsemine (Fig. 1, curve 1) contained

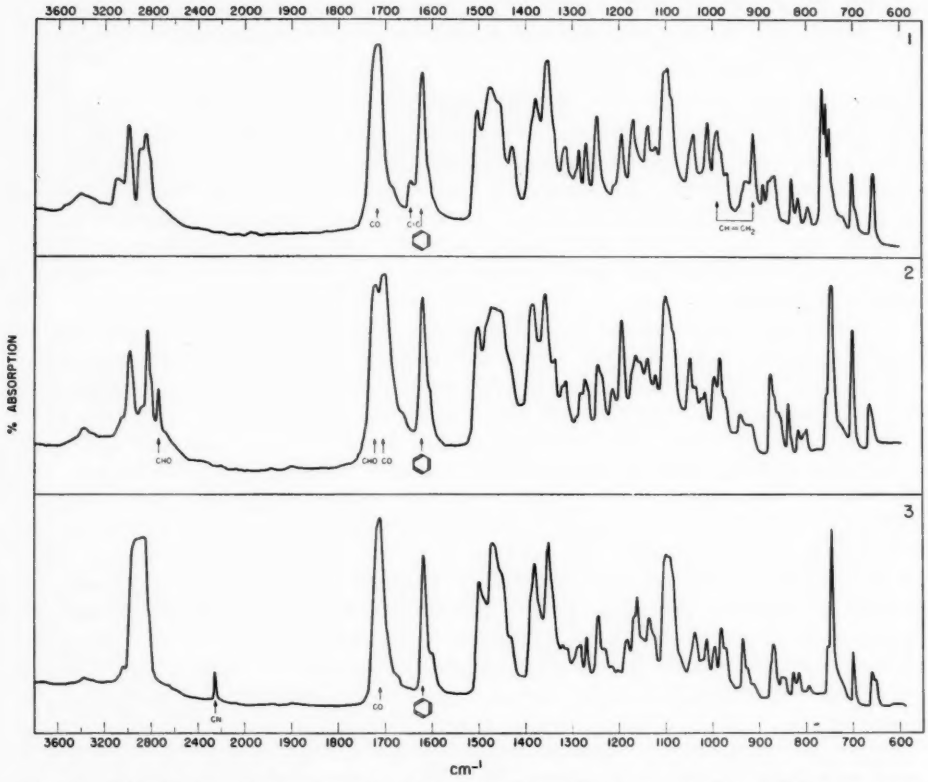


FIG. 1. Infrared absorption spectra of 1, N(a)-methylgelsemine; 2, the aldehyde derived from it; 3, the corresponding nitrile.

a band at 1702 cm.$^{-1}$ attributable to the oxindole carbonyl and bands at 911, 988, and 1645 cm.$^{-1}$ due to the olefinic linkage. The infrared absorption spectrum of the oxidation product (Fig. 1, curve 2) contained two bands in the carbonyl region, at 1702 cm.$^{-1}$ and at 1719 cm.$^{-1}$, attributable one to the oxindole carbonyl, the other to a newly formed carbonyl; further, there were no bands corresponding to those of an olefinic linkage,[5] but there was a sharp peak at 2735 cm.$^{-1}$ which is characteristic of the C—H stretching of an aldehyde group.

[4]*A preliminary account of the results has been published in J. Am. Chem. Soc.* **78**, *5127 (1956).*

[5]*As in the spectra of other dihydro derivatives of gelsemine, however, there was an unaccountable absorption band at ca. 990 cm.*$^{-1}$*, i.e., a double peak at 982–994 cm.*$^{-1}$.

Chemical evidence that the oxidation product ($C_{20}H_{22}O_3N_2$) was an aldehyde was soon forthcoming. The product was converted into an oxime ($C_{20}H_{23}O_3N_3$) which, on treatment with acetic anhydride and pyridine for several hours, yielded the corresponding nitrile ($C_{20}H_{21}O_2N_3$). The infrared absorption spectrum of this nitrile (Fig. 1, curve 3) contained a band at 1711 cm.$^{-1}$ due to the oxindole carbonyl, and a very sharp band at 2255 cm.$^{-1}$ characteristic of the C≡N vibration. The production of a nitrile under such conditions shows undoubtedly that the oxime was derived from an aldehyde.

Additional proof of the aldehyde nature of the oxidation product was obtained by its Wolff-Kishner reduction. This reaction gave rise to a compound ($C_{20}H_{24}O_2N_2$) which, by the Kuhn-Roth determination, was shown to contain a C—CH_3 group, whereas no such group could be detected in the oxidation product. In this reaction, such a group could be generated only by reduction of an aldehyde. The infrared absorption spectrum of the Wolff-Kishner reduction product ($C_{20}H_{24}O_2N_2$) showed a band at 1707 cm.$^{-1}$ due to the oxindole carbonyl, but the second carbonyl band and the band at 2735 cm.$^{-1}$ present in the spectrum of the oxidation product had disappeared.

It is possible, therefore, to conclude unequivocally that the readily reducible bond in gelsemine is present in a vinyl side chain.

A comparison of the basic strengths of the various products described with those of N(a)-methylgelsemine and dihydrogelsemine is revealing. There is a drop of pK_a in going from N(a)-methylgelsemine (7.25) to the aldehydic compound (6.15) of 1.1 units, and in going from the latter to the nitrile (4.95) of a further 1.2 units. On the other hand, the Wolff-Kishner reduction of the aldehyde gives a product with a pK_a of 7.65, which is the same as that of dihydrogelsemine. The marked effect on the basic strength caused by changing the vinyl group first to an aldehyde, then to a nitrile, and finally to a methyl shows that the vinyl side chain must be located relatively close to the basic nitrogen N(b).

EXPERIMENTAL

All infrared absorption spectra were taken with nujol mulls on a Perkin-Elmer double beam spectrophotometer, model 21B, using a sodium chloride prism. The specific rotations were measured in chloroform solutions and the pK_a values determined in 80% ethanol solutions.

Oxidation of N(a)-Methylgelsemine

N(a)-Methylgelsemine was prepared from gelsemine as described by Goutarel, Janot, Prelog, and Sneeden (4), m.p. 140–141°, pK_a 7.25. A solution of N(a)-methylgelsemine (2.0 g.) in dioxane (50 ml.) and water (15 ml.) was stirred with osmium tetroxide (50 mg.) and after half an hour finely powdered sodium metaperiodate (2.65 g.) was added to the dark mixture in small lots in the course of a half-hour (6). The solution became pale yellow and a colorless precipitate separated. Stirring was continued for 1.5 hours and water (100 ml.) was added. This produced a clear solution which was extracted with chloroform and the extract evaporated to dryness. It left a black residue which was redissolved in chloroform. This solution was extracted with hydrochloric acid, and the extract made basic and extracted with chloroform. This last extract yielded a basic fraction that crystallized readily from a mixture of acetone and *n*-hexane as colorless plates, wt. 1.3 g., m.p. 192–194°. A further quantity (0.2 g.) of less pure material, m.p. 186–190°, was obtained from the mother liquors. An analytical sample was recrystallized twice from the same mixture, m.p. 192–194°, $[\alpha]_D$ −16.3°±1.4° (*c*, 2.09), pK_a 6.15. Found: C, 71.10; H, 6.62; N, 8.53; N.CH_3, 8.61%. Calc. for $C_{20}H_{22}O_3N_2$: C, 70.98;

H, 6.55; N, 8.28; 2N.CH_3, 8.88%. A Kuhn-Roth determination of C—CH_3 was nil. Infrared, Fig. 1, curve 2.

Preparation of an Oxime

Hydroxylamine hydrochloride (150 mg.) in methanol (15 ml.) and water (5 ml.) was added to a solution of the aldehyde described above (250 mg.) in methanol (10 ml.), and the clear solution was heated under reflux in the presence of suspended sodium bicarbonate (400 mg.) for 18 hours. Evaporation to dryness left a residue, the chloroform soluble portion of which separated from methanol as colorless prismatic needles, wt. 224 mg., m.p. 267–268°. Recrystallization from the same solvent did not improve the melting point. Found: C, 68.21; H, 6.75; N, 11.81%. Calc. for $C_{20}H_{23}O_3N_3$: C, 67.97; H, 6.56; N, 11.89%.

Dehydration of the Oxime to a Nitrile

A solution of the oxime (500 mg.) in acetic anhydride (15 ml.) and pyridine (0.5 ml.) was kept at 100° for 18 hours. The solvent was removed under reduced pressure and the residue chromatographed on alumina (Woelm, Grade III, neutral, 30 g.) from a benzene solution. The product was eluted with benzene–ether mixtures. It separated from a mixture of acetone and *n*-hexane as colorless plates, wt. 134 mg., m.p. 238–240°. After two recrystallizations from the same mixture, it melted at 240–241°, $[\alpha]_D$ $-41.0\pm0.5°$ (*c*. 2.05), pK_a 4.95. Found: C, 71.57; H, 6.42; N, 12.49%. Calc. for $C_{20}H_{21}O_2N_3$: C, 71.62; H, 6.31; N, 12.53%. Infrared, Fig. 1, curve 3.

Further elution of the alumina column with methanol gave a substance which crystallized from methanol as prismatic needles, wt. 175 mg., m.p. 267–268° either alone or in admixture with the oxime.

Wolff-Kishner Reduction of the Aldehyde

A solution of the aldehyde (500 mg.) in triethylene glycol (10 ml.) and 85% hydrazine hydrate (5 ml.) was heated under reflux at 140° for 2 hours. Sodium hydroxide (500 mg.) was added and the mixture kept at 165° for 1 hour. The condenser was removed, and the temperature raised further to 205° and maintained for 4 hours. The basic material obtained from a chloroform extract of the cooled reaction mixture crystallized from acetone as colorless prismatic needles, wt. 124 mg., m.p. 172–174°. After two recrystallizations from the same solvent, the product melted at 173–175°. $[\alpha]_D$ $+12.1\pm1.0°$ (*c*, 2.06), pK_a 7.65. Found: C, 74.19; H, 7.56; N, 8.79; C—CH_3, 4.11%. Calc. for $C_{20}H_{24}O_2N_2$: C, 74.04; H, 7.46; N, 8.64; 1C—CH_3: 4.63%.

ACKNOWLEDGMENT

We are particularly indebted to Dr. R. U. Lemieux, University of Ottawa, who with Mr. J. P. Barrette carried out the oxidation of dihydrogelsemine to propionic acid, and to Dr. W. G. Schneider of these Laboratories for the Nuclear Magnetic Resonance spectra and their interpretation. We also express our gratitude to Mr. R. Lauzon for recording the infrared spectra.

REFERENCES

1. Chu, T. T. and Chou, T. Q. J. Am. Chem. Soc. **62**, 1955 (1940).
2. Gibson, M. S. and Robinson, R. Chemistry & Industry, **93** (1951).
3. Goutarel, R., Janot, M. M., Prelog, V., Sneeden, R. P. A., and Taylor, W. I. Helv. Chim. Acta, **34**, 1139 (1951).
4. Goutarel, R., Janot, M. M., Prelog, V., and Sneeden, R. P. A. Helv. Chim. Acta, **34**, 1962 (1951).
5. Lemieux, R. U. and Purves, C. B. Can. J. Research, B, **25**, 485 (1947).
6. Pappo, R., Allen, D. S., Jr., Lemieux, R. U., and Johnson, W. S. J. Org. Chem. **21**, 478 (1956).

FREE RADICALS BY MASS SPECTROMETRY

XII. PRIMARY STEPS IN THE MERCURY PHOTOSENSITIZED DECOMPOSITIONS OF ACETONE AND ACETALDEHYDE[1]

F. P. LOSSING

ABSTRACT

The primary step in the mercury (Hg^3P_1) photosensitized decomposition of acetone at 55° C. results in the formation of methyl and acetyl radicals. At least 25% of the acetyl radicals are sufficiently long-lived to suffer collision with a second excited mercury atom, the products being ketene and a hydrogen atom. The primary step in the decomposition of acetaldehyde is at least 95% to form methyl and formyl radicals. The methane found was shown by tracer experiments to be the product of a secondary reaction, probably that between methyl and formyl radicals. Other secondary reactions are discussed.

INTRODUCTION

Acetaldehyde

Although a great many studies have been made of the direct photolysis of acetaldehyde and acetone, very few experiments on the mercury photosensitized reactions of these compounds have been reported. Using the Paneth technique, the detection of radicals in the mercury photosensitized decomposition of acetaldehyde has been briefly described (23). Cvetanović (7) reported a few experiments on the mercury sensitized decomposition, the products being CO, CH_4, H_2, and C_2H_6. He concluded that CH_3 radicals were undoubtedly a product of the reaction. The quenching cross section of acetaldehyde for Hg^3P_1 atoms was found (6) to be 29 (relative to *n*-butane equal to 3.6). In the absence of other reported data it is of interest to review briefly the results of direct photolysis experiments. These results have been discussed recently by Steacie in some detail (21). Three primary processes have been proposed:

$$CH_3CHO + h\nu \rightarrow CH_3 + CHO, \quad [1]$$
$$CH_3CHO + h\nu \rightarrow CH_4 + CO, \quad [2]$$
$$CH_3CHO + h\nu \rightarrow CH_3CO + H. \quad [3]$$

There appears to be ample evidence that free radicals are produced in the reaction. Using the mirror technique, the presence of radicals in the direct photolysis has been clearly demonstrated. By this means, Pearson and Purcell (20) identified the radicals as methyl, and concluded that at 100° C. fewer methyl radicals were produced than from acetone under the same conditions. This would be consistent with a part of the reaction proceeding by reaction [2] although the calculation is probably not very reliable. Volman *et al.* (23) showed that radicals were produced at wavelengths from 2537 Å to 3130 Å.

A number of workers have studied the photolysis in the presence of iodine on the assumption that the ratio of methyl iodide to methane in the products would give the ratio of the quantum efficiencies of reactions [1] and [2]. In this way Gorin (11) found the sum of the quantum yields to be about 1 at 2537 Å and 3130 Å although the ratio of efficiencies changed from 1/2.9 at 2537 Å to 2.6/1 at 3130 Å. A re-examination of the photolysis in the presence of iodine by Blacet and co-workers (2, 3) led to the different conclusion that at 3130 Å $\Phi_{CO} \simeq \Phi_{CH_3I} = 0.20$ and $\Phi_{CH_4} \simeq 0.013$, and that reaction [1]

[1]*Manuscript received November 29, 1956.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 4238.

predominated. At shorter wavelengths Φ_{CH_3I} rose slightly and Φ_{CH_4} increased greatly, the latter becoming almost equal to Φ_{CH_3I} at 2380 Å. This work indicated a significant increase in the efficiency of reaction [2] at shorter wavelengths. Later work on the iodine inhibited photolysis of acetone (17) suggested that iodine may deactivate excited acetaldehyde molecules. This interpretation of the results of Buchanan (4) has been suggested by Steacie (21), who concluded that the interpretation of the results of photolysis in the presence of iodine becomes a matter of some complexity, and that these experiments do not give the relative efficiencies of reactions [1] and [2] in the absence of iodine.

From experiments using deuteroacetaldehyde, Zemany and Burton (24) concluded that free radicals were certainly produced, but that a small amount of decomposition into CH_4 and CO occurred as well. Blacet and Brinton (1), using CH_3CDO, showed that the isotopic composition of the methane produced approached that corresponding to the intramolecular reaction [2] as other methane-producing reactions were suppressed by iodine.

Recently, work by Calvert, Pitts, and Thompson (5) showed that at 3130 Å the limiting quantum yield of reaction [1] at high temperatures is about 0.8. They suggested that the quantum yield of 0.2 found for this process in the iodine-inhibited photolysis might correspond to an excited state of acetaldehyde which dissociated in one of its first vibrations, and hence would not be quenched by iodine. Other longer-lived states formed could lead to radical formation in the absence of iodine. Their results set an upper limit of about 0.20 for the primary efficiency of reaction [2].

From flash photolysis experiments, Khan, Norrish, and Porter (13) concluded that the products formed from acetaldehyde corresponded to $\leqslant 46\%$ of [1], 43% (or more) of [2], and 11% of [3].

On the whole there is considerable evidence that reaction [2] may occur to a significant extent in the photolysis at 2537 Å, although there is some question as to the interpretation of the results.

Acetone

A few experiments on the mercury photosensitized decomposition of acetone were described by Steacie and Darwent (22) from which they concluded that the mechanism was similar to that of the direct photolysis. The quenching cross section of acetone for Hg^3P_1 atoms was measured by Darwent, Phibbs, and Hurtubise (8) to be 32×10^{-16} cm.^{2}, almost the same as that of propylene (31×10^{-16} cm.^{2}) and slightly less than that of isobutene (37×10^{-16} cm.^{2}). Cvetanović (6) found a slightly higher value.

McNesby, Gordon, and Smith (16) reported that no hydrogen was formed in the mercury photosensitized decomposition, and that the possible primary step

$$CH_3COCH_3 + Hg^* \rightarrow H + CH_3COCH_2 + Hg \qquad [4]$$

could be ruled out.

Some preliminary experiments were reported recently from this laboratory (10) in which methyl and acetyl radicals were detected in the mercury photosensitized decomposition of acetone using mass spectrometry.

The direct photolysis of acetone has been the subject of a large number of publications and it appears to be well established that the primary step is entirely a free radical one (19, 21):

$$CH_3COCH_3 + h\nu \rightarrow CH_3 + CH_3CO. \qquad [5]$$

The lifetime of the acetyl radical at the shorter wavelengths may be quite short as a result of energy carry-over, and the primary step is then effectively

$$CH_3COCH_3 + h\nu \rightarrow 2CH_3 + CO. \quad [6]$$

There appears to be no evidence for intramolecular rearrangements leading to methane or ethane production.

Although the primary processes in mercury photosensitized reactions may not be the same as in the direct photolysis, it was thought that some evidence for the nature of the primary steps would be of interest, and that this might be obtained by direct observation of the radical and non-radical primary products by the use of a mass spectrometer. In particular the ratios of the free radical and the intramolecular rearrangement processes such as

$$CH_3CHO + Hg^* \rightarrow CH_3 + CHO + Hg, \quad [7]$$
$$CH_3CHO + Hg^* \rightarrow CH_4 + CO + Hg, \quad [8]$$

comparable to reactions [1] and [2], might be estimated.

EXPERIMENTAL

The photosensitized reactions were carried out in a reactor attached to a mass spectrometer, the apparatus having been described in detail in a previous publication (14). From the illuminated zone, the reaction products, including radicals, were allowed to enter the ionization chamber of the mass spectrometer and were identified by observation of the mass spectra using low electron energy. Quantitative measurements of the amounts of products formed were made using 50 volt electrons. The sensitivities of the instrument to the stable products and reactants were measured from standard samples using a Consolidated Electrodynamics Corp. micromanometer. Helium was added to the standard samples to bring the total pressure up to that used in the flow system during the decomposition experiments (1 cm. Hg). The sensitivity of methyl radicals was taken as 0.47 of that of methane, as measured previously (15). A correction was made for the formation of methane from methyl in the ionization chamber as was found necessary in earlier work (12). This correction was of importance in measuring the small amount of methane produced in the acetone reaction. In order to find out if this correction was of the same magnitude as found previously, some experiments were made in which CD_3COCD_3 was photodecomposed in the reactor. The methane produced was found to be about one-half CD_3H and the rest CD_4. After allowing for the small percentage of H atoms present in the sample of deuteroacetone, it was seen that the amount of CD_3H formed was almost exactly $0.1 \times [CH_3]$ as found earlier (12). This amount must be formed by abstraction from water absorbed on the walls of the ionization chamber. After passing CD_3COCD_3 through the reactor for 1 hour, the amount of CD_3H formed on illumination had decreased appreciably, presumably as a result of deuterization of the adsorbed water. On returning to CH_3COCH_3 a small temporary formation of CH_3D was observed. This experiment shows that the correction used for the methane production was of the right magnitude.

The Reactor and Its Operation

A schematic representation of the reactor is given in Fig. 1. For convenience, the axis of the reactor is shown as being horizontal rather than vertical. The reactant, at a partial

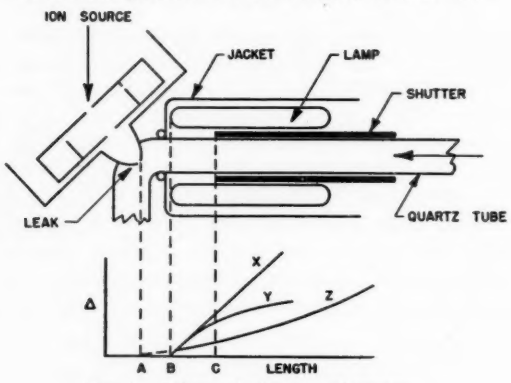


FIG. 1. Schematic diagram of reactor.

pressure of less than 10 microns, was carried in a stream of helium saturated* with mercury vapor at 55° C. The extent of decomposition and the composition of the products were determined by comparing the mass spectra obtained in the presence and absence of irradiation. In order to aid in distinguishing between primary and secondary products, use was made of a movable shutter surrounding the reactor. The length of the illuminated zone, and consequently the contact time, could in this way be varied conveniently. The relation between the extent of reaction (Δ) and the length of the illuminated zone can be understood by reference to Fig. 1. The concentrations of reactant and products are, of course, those existing at the tip of the leak (point A). With the end of the shutter at position B, a small amount of 2537 Å radiation could enter the reactor obliquely between the shutter and the water jacket. For this reason a small amount of reaction was found to occur when the measured length of the illuminated zone was zero. As the shutter is withdrawn toward position C, the amount of reactant decomposed should follow an almost linear relation with length, as shown by the line X. The concentration of a radical might be expected to follow a relation roughly like that of line Y, its shape depending on the mechanism of removal of the radical. This line could be above the line X for part of the course if, as in acetone, two methyl radicals were ultimately produced for one molecule decomposed. Both of these lines should extrapolate roughly to the point B. The product of the combination of radicals should follow a relation such as that shown by line Z. However, the contact time available for combination and abstraction steps is not that corresponding to the length BC, but that corresponding to the length AC. For this reason the line Z may not extrapolate to point B for fast bimolecular reactions but would have an apparent origin at point A. The interpretation of the data would have been simplified had it been possible to design a reactor in which the distance AB was zero. Owing to the considerable dimensions of the movable water jacket and the ion source, it was not possible to make the distance AB less than 1.5 cm. with the design used.

At the flow rate used in this work, the contact time corresponding to a 10 mm. length of reactor was 0.34 millisecond at 55° C.

Some difficulty was encountered, in the case of acetone, with the formation of an opaque deposit on the walls of the reactor. This caused a decrease in the extent of de-

**The completeness of saturation was doubtful.*

composition during the time taken to scan the spectrum of the reaction products. To avoid this difficulty the partial pressure of acetone had to be reduced to less than 2μ, at which pressure the effect was not too serious.

Materials

The acetone and acetaldehyde used were Eastman Kodak White Label products. After these were thoroughly outgassed, the mass spectra showed no sign of impurities. The acetone-d_6 was supplied by Merck and Company (Canada). From the mass spectrum the sample was found to be 98 mole % of acetone-d_6. The deuteroacetaldehyde was prepared by Dr. L. C. Leitch. It contained 91 mole % of CD_3CDO. The standard samples used for sensitivity measurements were cylinder gases purified where necessary. The ketene standard was the gift of Mr. J. Shoosmith.

RESULTS AND DISCUSSION

Acetone

Using low energy electrons for detecting radicals (9), methyl radicals in considerable abundance were found among the products of the mercury photosensitized decomposition of acetone. With higher pressures of acetone, acetyl radicals (mass 43) and biacetyl (mass 86) were also detectable (10). Other products identified using low energy electrons were carbon monoxide, ethane, ketene, and a trace of methane. The amounts of acetone decomposed and of products formed with the shutter in different positions were determined from the 50 volt mass spectra. These data are given in Table I. The low carbon

TABLE I

DECOMPOSITION OF ACETONE

Acetone (μ)		Length of illuminated zone (mm.)	Acetone decomposed		Products (μ)						Balance (%) of:		
Lamp off	Lamp on		%	μ	CO	CH_4	CH_3	C_2H_6	CH_2CO	H_2	O	C	H
1.61_0	1.24_3	36	22.8	0.36_7	0.25_0	0.01_8	0.16_5	0.16_7	0.08_0	0.08_4	90	84	86
1.61_0	1.40_2	18	12.9	0.20_8	0.15_1	0.00_4	0.13_8	0.06_1	0.05_2	0.04_2	98	84	79
1.61_0	1.48_8	9	7.5	0.12_2	0.09_0	0.00_0	0.11_0	0.02_4	0.03_1	0.02_8	99	85	81

and oxygen balances probably result from the formation of the solid material mentioned above. At this pressure of acetone, the amounts of acetyl radicals and biacetyl detected were negligibly small.

The change in composition of the products as a function of the length of the illuminated zone can be seen from Fig. 2. The lines for acetone decomposed and for CO formed do not extrapolate to the measured zero position (*B*, Fig. 1) but to a position 2 or 3 mm. below it. This may be a result of scattered or reflected light entering the reactor below point *B*. It is evident that methyl radicals and CO are the major primary products and that the small amount of methane is produced in a secondary reaction. The presence of significant amounts of ketene and hydrogen is interesting, as they are not found in the direct photolysis. The following modes of formation of ketene may be considered:

$$CH_3COCH_3 + Hg^* \rightarrow CH_4 + CH_2{=}CO + Hg, \quad [9]$$

$$CH_3 + CH_3CO \rightarrow CH_4 + CH_2{=}CO, \quad [10]$$

$$2CH_3CO \rightarrow CH_2{=}CO + CH_3CHO, \quad [11]$$

$$CH_2CO \rightarrow CH_2{=}CO + H, \quad [12]$$

$$CH_3CO + Hg^* \rightarrow CH_2{=}CO + H + Hg. \quad [13]$$

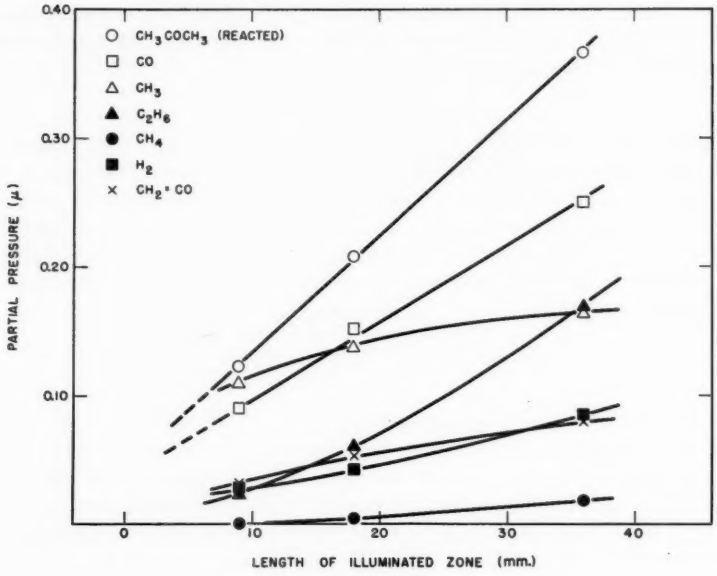


FIG. 2. Decomposition of acetone.

Reactions [9] and [10] can at once be eliminated as a source of ketene since the methane yield is too small. The absence of acetaldehyde as a product rules out reaction [11]. The thermal decomposition of the acetyl radical is known to proceed only by

$$CH_3CO \rightarrow CH_3 + CO \qquad [14]$$

rather than by reaction [12], the quantum yield of CO in the direct photolysis above 100° C. being 1 within the experimental error (19). The remaining possibility, reaction [13], is not improbable under conditions of high concentrations of excited mercury atoms such as exist in the present experiments. A similar reaction between radicals and excited atoms appeared to occur in the photosensitized decomposition of propylene and the butenes (14). Reaction [13] would also explain the formation of hydrogen, which was not a product under the conditions existing in the experiments of McNesby *et al.* (16). It should be noted that the hydrogen yield is approximately twice what would be expected on the yield of one atom per ketene formed. At these low concentrations of H atoms it is possible that all the molecular hydrogen arises from a reaction in the ion source between H atoms and water adsorbed on the walls of the ionization chamber. A similar reaction of methyl radicals is discussed above.

If reaction [13] can be taken as the origin of the ketene, then each ketene molecule represents an acetyl radical which has survived sufficiently long to collide with a second excited mercury atom. In the three experiments given in Table I, the ratio of ketene to acetone decomposed is respectively 0.22, 0.25, and 0.25. Evidently at least 25% of the acetyl radicals formed in the primary step do not carry over sufficient energy to dissociate immediately. Since the amounts of biacetyl and acetyl detected were small,

the remaining 75% of the acetyl radicals formed must dissociate to form $CH_3 + CO$ by one or more of the three possible modes:

$$CH_3CO^* \rightarrow CH_3 + CO, \quad [15]$$
$$CH_3CO \rightarrow CH_3 + CO, \quad [14]$$
$$Hg^* + CH_3CO \rightarrow CH_3 + CO + Hg. \quad [16]$$

Noyes and Dorfman (18) concluded that in the direct photolysis of acetone at 2537 Å, 22% of the acetyl radicals formed dissociated immediately by reaction [15]. Since the cross section for reaction [16] is unknown and the rate of reaction [14] at such low pressures is uncertain, the present data do not provide an estimate of the importance of reaction [15] in the mercury photosensitized decomposition of acetone.

The following reactions would account for the composition of the products:

$$CH_3COCH_3 + Hg^* \rightarrow CH_3 + CH_3CO + Hg, \quad [17]$$
$$2CH_3 \rightarrow C_2H_6, \quad [18]$$
$$CH_3 + H + M \rightarrow CH_4 + M, \quad [19]$$
$$Hg^* + CH_3CO \rightarrow CH_2{=}CO + H + Hg, \quad [13]$$
$$CH_3CO \rightarrow CH_3 + CO, \quad [14]$$
$$H + (H \text{ adsorbed}) \rightarrow H_2. \quad [20]$$

Two other reactions leading to the removal of acetyl radicals may also occur:

$$Hg^* + CH_3CO \rightarrow CH_3 + CO + Hg, \quad [16]$$
$$CH_3CO^* \rightarrow CH_3 + CO. \quad [15]$$

There is no evidence for alternative primary steps such as reactions [4] or [9]. This is in agreement with the findings of McNesby *et al.* (16).

Acetaldehyde

Using low electron energies, methyl radicals were found in abundance as a product of the decomposition of acetaldehyde. With higher pressures of acetaldehyde ($>10\mu$) a small increase in the mass 29 peak was detected and may be attributable to the CHO radical. The formation of ethane in large amounts caused interference at mass 29 and decreased the sensitivity available for the detection of CHO. The appearance of a parent peak at mass 58, although not positively identified as that of glyoxal, the dimer of CHO, also suggested strongly that the CHO radical was present.

From a comparison of the 50 volt mass spectra obtained with and without illumination, the amount of decomposition of acetaldehyde and the composition of the products were obtained. The results obtained with different contact times are given in Table II. With

TABLE II

DECOMPOSITION OF ACETALDEHYDE

Acetaldehyde (μ)		Length of illuminated zone (mm.)	Acetaldehyde decomposed		Products (μ)					Balance (%) of:		
Lamp off	Lamp on		%	μ	CO	CH_4	CH_3	C_2H_6	H_2	O	C	H
5.91_0	4.01_0	36	32.1	1.90	1.81	0.58_0	0.09_1	0.53_6	0.62	96	93	93
5.88_0	4.96_2	18	15.6	0.91_8	0.90_1	0.28_2	0.12_1	0.21_5	0.27	98	94	90
5.85_3	5.31_5	9	9.2	0.53_8	0.48_2	0.12_0	0.21_2	0.10_6	0.10	90	96	91
5.82_5	3.98_9	36	31.5	1.84	1.73	0.57_2	0.15_4	0.53_9	0.60	94	96	98
5.79_8	4.86_4	18	16.1	0.93_4	0.93_8	0.28_3	0.16_2	0.23_2	0.27	100	99	95
5.85_3	5.37_1	9	8.2	0.48_2	0.48_4	0.11_8	0.18_7	0.09_9	0.10	100	102	95

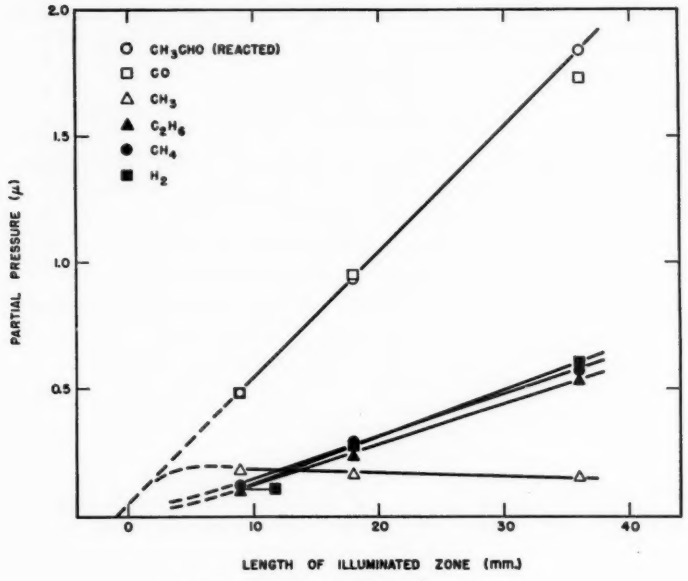


FIG. 3. Decomposition of acetaldehyde.

the low pressures of acetaldehyde used, the CHO and $(CHO)_2$ concentrations were negligible, and the products listed give fairly complete carbon and oxygen balances. The relation between the composition of the products and the contact time is shown in Fig. 3. The yield of CO was unity within the experimental error, and it is evident that a primary reaction to produce methyl radicals predominated. However, the yield of methane was much higher than was found for acetone. Either methane was produced in the primary step, or it was formed by a fast bimolecular reaction, such as

$$CH_3 + CHO \rightarrow CH_4 + CO. \qquad [21]$$

The disappearance of CHO by thermal decomposition or by collision with an excited mercury atom would lead to the formation of hydrogen and CO, but it can be seen that the yield of H_2 is much less than one-half the yield of CO. Reaction [21] must therefore be considered as a possible source of methane.

In order to determine how much of the methane arose from reaction [21] some experiments were carried out in which CD_3CDO was decomposed in the presence of varying amounts of CH_3CHO. If the methane were produced in a primary step, the addition of CH_3CHO should not affect the yield of CD_4 from CD_3CDO. For these experiments the length of the reaction zone was 18 mm. The results are given in Table III. In the absence of CH_3CHO some CD_3H was formed, partly as a result of the 9% of acetaldehyde which contained one protium atom, and partly as a result of the reaction of CD_3 with adsorbed water as described earlier. This fraction should be approximately constant and should not affect the yield of CD_4 to any great extent. As can be seen from Table III, the yield of CD_4 per molecule of CD_3CDO decomposed, although almost independent of the partial pressure of CD_3CDO in the absence of CH_3CHO, decreased significantly when CH_3CHO was added. The yield (CD_4 formed/CD_3CDO decomposed) is shown in Fig. 4 plotted

TABLE III

CD_4 PRODUCTION IN CD_3CDO–CH_3CHO MIXTURES

$CD_3CDO(\mu)$, initial	$CH_3CHO(\mu)$, initial	CD_3CDO decomposed		CH_3CHO decomposed		CD_4 formed (μ)	CD_3H formed (μ)	CD_4 formed / CD_3CDO decomposed
		μ	%	μ	%			
11.13	—	1.19	10.7	—	—	0.37	0.08	0.31
5.38	—	0.64	11.9	—	—	0.17	0.04	0.27
5.23	5.86	0.63	12.0	0.67	11.4	0.095	0.10_5	0.15
3.06	—	0.43	14.1	—	—	0.13_1	0.023	0.30
3.06	1.58	0.42	13.7	0.25	15.8	0.089	0.082	0.21
3.06	3.86	0.41	13.4	0.50	13.0	0.066	0.11_5	0.16
3.06	5.56	0.43	14.1	0.83	14.9	0.046	0.089	0.11
3.06	9.42	0.39	12.7	1.26	13.4	0.030	0.082	0.07_7

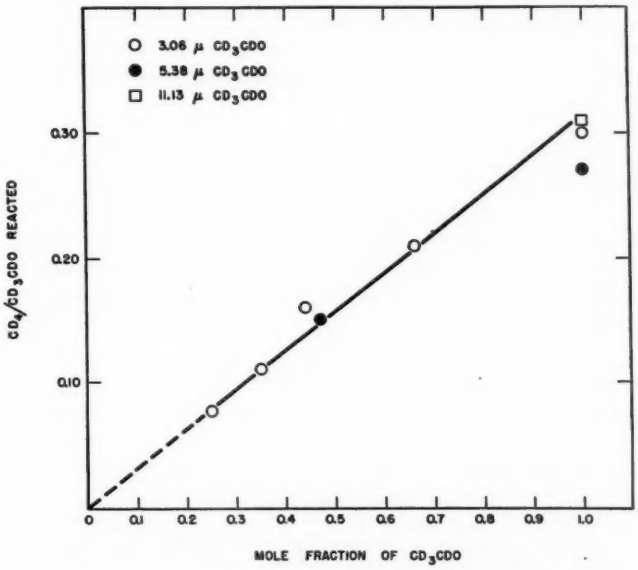


FIG. 4. CD_4 production from CD_3CDO–CH_3CHO mixtures.

against the mole fraction of CD_3CDO. Within the experimental error the line can be extrapolated to the origin. This indicates that the methane is not produced in the primary step, but by a bimolecular process. The most probable process is reaction [21], which, to explain the yield curves given in Fig. 3, would have to be at least as fast as the combination of methyl radicals at this pressure. The alternative reaction between methyl and formyl,

$$CH_3 + CHO \rightarrow CH_3CHO, \qquad [22]$$

appeared to be slow at these pressures, since only a very slight formation of CD_3CHO and CH_3CDO occurred when the mixture was illuminated. The formation of hydrogen shows that a considerable fraction of the CHO radicals disappeared by one or both of the following reactions:

$$CHO \rightarrow CO + H, \qquad [23]$$

$$Hg^* + CHO \rightarrow CO + H + Hg. \qquad [24]$$

As in the case of acetyl, the present data do not allow a calculation of the relative importance of these two reactions.

The probable error in the data shown in Fig. 4 is difficult to estimate, but these results would indicate that less than 0.05 of the primary step proceeds by the intramolecular rearrangement to form methane and carbon monoxide.

CONCLUSION

From these experiments it appears that the reaction of Hg^3P_1 atoms with acetone results in a dissociation into methyl and acetyl radicals, similar to the direct photolysis at 2537 Å. One-quarter of the acetyl radicals formed in this step do not dissociate immediately but react with a second excited mercury atom to form ketene and a hydrogen atom. Since other reactions removing acetyl are probable, it is evident that considerably more than one-quarter of the radicals survive the primary act. This is not inconsistent with a fraction of 0.78 surviving the primary act in the direct photolysis (18).

The reaction of acetaldehyde with Hg^3P_1 atoms leads to a dissociation into methyl (and presumably formyl) radicals to the extent of at least 95%. The methane found as a product results from the reaction

$$CH_3 + CHO \rightarrow CH_4 + CO. \qquad [21]$$

This reaction must be at least as fast at 55° C. and low pressures as the combination of methyl radicals to form ethane. Owing to experimental uncertainties, a molecular rearrangement comprising 5% or less of the primary act cannot be ruled out. This limit for the rearrangement process is considerably lower than has been suggested in the direct photolysis at 2537 Å, but recent work at 3130 Å (5) raises some doubts as to the reliability of the earlier data.

ACKNOWLEDGMENT

The author wishes to thank Dr. K. O. Kutschke and Dr. R. J. Cvetanović for many helpful discussions.

REFERENCES

1. Blacet, F. E. and Brinton, R. K. J. Am. Chem. Soc. **72**, 4715 (1950).
2. Blacet, F. E. and Heldman, J. D. J. Am. Chem. Soc. **64**, 889 (1942).
3. Blacet, F. E. and Loeffler, D. E. J. Am. Chem. Soc. **64**, 893 (1942).
4. Buchanan, A. S. J. Chem. Soc. 2317 (1951).
5. Calvert, J. G., Pitts, J. N., and Thompson, D. D. J. Am. Chem. Soc. **78**, 4239 (1956).
6. Cvetanović, R. J. J. Chem. Phys. **23**, 1208 (1955).
7. Cvetanović, R. J. Can. J. Chem. **34**, 775 (1956).
8. Darwent, B. de B., Phibbs, M. K., and Hurtubise, F. G. J. Chem. Phys. **22**, 859 (1954).
9. Eltenton, G. C. J. Chem. Phys. **15**, 455 (1947).
10. Farmer, J. B., Lossing, F. P., Marsden, D. G. H., and Steacie, E. W. R. J. Chem. Phys. **23**, 1169 (1955).
11. Gorin, E. Acta Physicochim. U.R.S.S. **9**, 681 (1938); J. Chem. Phys. **7**, 256 (1939).
12. Ingold, K. U. and Lossing, F. P. J. Chem. Phys. **21**, 1135 (1953).
13. Khan, M. A., Norrish, R. G. W., and Porter, G. Proc. Roy. Soc. (London), A, **219**, 312 (1953).
14. Lossing, F. P., Marsden, D. G. H., and Farmer, J. B. Can. J. Chem. **34**, 701 (1956).
15. Lossing, F. P. and Tickner, A. W. J. Chem. Phys. **20**, 907 (1952).
16. McNesby, J. R., Gordon, A. S., and Smith, S. R. J. Am. Chem. Soc. **78**, 1287 (1956).
17. Martin, G. R. and Sutton, H. C. Trans. Faraday Soc. **48**, 812 (1952).
18. Noyes, W. A. and Dorfman, L. M. J. Chem. Phys. **16**, 788 (1948).
19. Noyes, W. A., Porter, G. B., and Jolley, J. E. Chem. Revs. **56**, 49 (1956).
20. Pearson, T. G. and Purcell, R. H. J. Chem. Soc. 1151 (1935).
21. Steacie, E. W. R. Atomic and free radical reactions. 2nd ed. Reinhold Publishing Corporation, New York. 1954.
22. Steacie, E. W. R. and Darwent, B. de B. J. Chem. Phys. **16**, 230 (1948).
23. Volman, D. H., Leighton, P. A., Blacet, F. E., and Brinton, R. K. J. Chem. Phys. **18**, 203 (1950).
24. Zemany, P. D. and Burton, M. J. Phys. & Colloid Chem. **55**, 949 (1951); J. Am. Chem. Soc. **73**, 499 (1951).

HYDROGENOLYSIS OF CARBOHYDRATES

II. REDUCTION OF METHYL α-D-GLUCOPYRANOSIDE[1]

E. VON RUDLOFF, D. E. STUETZ,[2] AND H. F. BAUER[3]

ABSTRACT

The hydrogenolysis of methyl α-D-glucopyranoside at elevated temperatures and pressures with copper chromite as catalyst and anhydrous dioxane as solvent was studied. The reaction produced a complex mixture of alcohols, the major components being 2-hydroxymethyl-4,5-dihydroxytetrahydropyran, methanol, isomeric hexanediols, 1,2-propanediol, and ethanediol.

A previous communication (1) describes the hydrogenolysis of methyl β-L-arabopyranoside using copper chromium oxide as catalyst and dioxane as solvent. At 240° C. and 250 atmospheres pressure *cis*- and *trans*-3,4-dihydroxytetrahydropyran were the major reaction products, showing that the carbon skeleton of methyl arabopyranoside is relatively stable to hydrogenolysis conditions necessary to cleave the carbon–oxygen bonds of free hydroxyl groups.

The investigation has now been extended to a study of the reaction of hydrogen with methyl α-D-glucopyranoside under the same reaction conditions. The hydrogenolysis proceeded readily, and after 3 hours' reaction time very little starting material could be detected. The product, unlike that in the reaction with methyl arabopyranoside however, was a complex mixture consisting of about 20% 2-hydroxymethyl-4,5-dihydroxytetrahydropyran (I); 10% isomeric hexanediols; 7% 1,2-propanediol; 5% ethanediol; smaller amounts of glycerol, *n*-hexanol, and unidentified compounds; 21% water; and 13% methanol. These results clearly indicate that the cyclic skeleton of glucopyranoside is considerably less resistant to reductive degradation than that of arabopyranoside.

Zartman and Adkins (15) have studied the reaction of hydrogen with methyl α-D-glucopyranoside using ethanol as solvent and slightly higher temperatures and pressures than in the present work. These authors fractionally distilled the reaction product *in vacuo* with a Widmer column and obtained, besides water, methanol, and ethanol, about 15% 1,2-propanediol, 9% hexanetriol, 7.5% hexanetetrol, and 5% of a compound tentatively identified as 2-(4-hydroxytetrahydrofuryl)-methyl carbinol, leaving about 25% unaccounted for. In view of the difficulties encountered during the present work in the separation of mixed polyols by fractional distillation, it is possible that some of the fractions obtained by Zartman and Adkins were mixtures. The finding of a tetrahydrofurfuryl alcohol as the only cyclic product is surprising, but may be due to the use of ethanol as solvent. Also, it is conceivable that under the somewhat more drastic conditions used by these authors, tetrahydropyran alcohols are extensively degraded.

Fractional distillation *in vacuo* of the reaction product obtained after evaporation of the dioxane did not give a clear-cut separation of the components, but 2-hydroxymethyl-4,5-dihydroxytetrahydropyran and small amounts of *n*-hexanol and *meso*-3,4-hexanediol could be isolated in a pure state. At temperatures above 160° C. decomposition or interaction between the polyols appeared to occur. To overcome this difficulty,

[1]*Manuscript received December 10, 1956.*
Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan. (Part I. J. Am. Chem. Soc. **78**, *4097 (1956).)*
Issued as N.R.C. No. 4247 and as Paper No. 240 on the Uses of Plant Products.
[2]*Present address: Celanese Corporation of America, Research Laboratories, Summit, New Jersey.*
[3]*Present address: E. I. DuPont de Nemours & Co. (Inc.), Photo Products Department, Parlin, New Jersey.*

the hydrogenolysis product was acetylated and then fractionally distilled at 8 mm. pressure in a 28 mm. Podbielniak column. Although a large number of fractions was collected, none could be shown, by paper chromatography, to consist of a single compound. Nearly all fractions contained varying amounts of 1,2-propanediol, ethanediol, and glycerol, indicating that azeotrope formation had occurred. Acetates are known to form azeotropic mixtures, but azeotrope formation by mixtures of acetates of diols and glycerol appear not to have been reported previously (8). Many neighboring fractions were found to contain the same components in varying amounts and were, therefore, combined to give the 12 fractions described in Table I. The compounds reported

TABLE I

FRACTIONS OBTAINED FROM THE ACETYLATED HYDROGENOLYSIS PRODUCT (1000 G.)

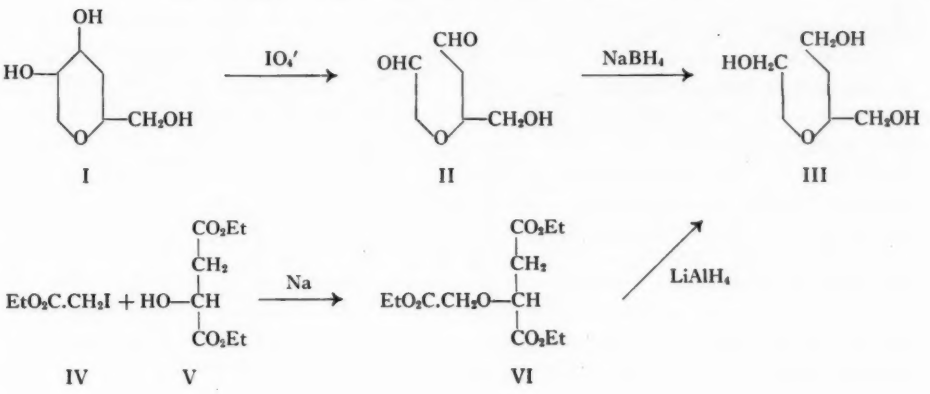
Fraction number	Boiling point at 12 mm. (°C.)	n_D^{20}	% of total hydrogenolysis product
1	60–62	1.4062	1.2
2	62–82	1.4280	2.9
3	82–84	1.4206	4.1
4	84–88	1.4243	1.6
5	88–96	1.4288	5.1
6	96–103	1.4241	2.4
7	103–115	1.4267	3.2
8	115–117	1.4285	3.3
9	117–147	1.4423	5.8
10	147–149	1.4529	3.7
11	149–176	1.4377	9.2
12	176–186	1.4571	6.4
Residue	(186)	(1.4575)	11.2

above were obtained from these combined fractions by solvent segregation and redistillation of the free alcohols. 1,2-Propanediol, ethanediol, and glycerol were determined by the chromatographic procedure of Dal Nogare (3). Water and methanol were determined as previously described (1) before evaporation of the dioxane. Some material appears to be lost during evaporation of the solvent and also during distillation steps, indicating that volatile products other than water, methanol, and dioxane having boiling points below that of *n*-hexanol may be present.

On determining the methoxyl content of various fractions high values were obtained even for those where no methoxyl content was expected. It was discovered subsequently that some of the free polyols, including ethane-, 1,2-propane-, and 2,3-butanediols as well as glycerol, give high values (13) for methoxyl content using the standard analytical procedure (7). This procedure, therefore, cannot be applied in the determination of methyl ethers when aliphatic polyols are present.

The structure of 2-hydroxymethyl-4,5-dihydroxytetrahydropyran (I) was determined as follows: the triol was oxidized with periodic acid and the resulting dialdehyde (II) was reduced with sodium borohydride to give an open-chain triol ether (III). This triol ether was synthesized by reaction of ethyl iodoacetate (IV) with L-malic acid diethyl ester (V) in the presence of sodium, and subsequent reduction of the resulting triester (VI) with lithium aluminum hydride.

The open-chain α-diols and glycerol were characterized by periodic acid oxidation and identification of the aldehydes thus obtained. Glycerol was further characterized by



Feigl's spot test (5). Of the isomeric hexanediols, *meso*-3,4-hexanediol could be obtained in a pure state by recrystallization. The liquid DL-3,4-hexanediol presented more difficulty and it is doubtful whether the compound finally analyzed was completely free from the meso isomer. 1,4-Hexanediol, also a liquid, was obtained free from α-glycols by destroying the latter with periodate.

As was noticed previously (1) when the starting material contained reducing material in excess of about 0.03% (calculated as glucose) as determined with Somogyi's reagent, the catalyst sometimes suffered decomposition. The technical methyl α-D-glucopyranoside initially used (0.46% reducing value calculated as glucose) was, therefore, recrystallized several times from methanol or ethanol until a satisfactory product was obtained.

EXPERIMENTAL

All melting points are corrected and were measured with a Leitz heating stage microscope. Fractional distillation was carried out with a Podbielniak packed (28 mm.) or a Podbielniak spinning band (8 mm.) column. The determinations of water, methanol, reducing values with Somogyi's reagent, and infrared spectra were carried out in the same manner as previously described (1).

Paper chromatograms were obtained by using Whatman No. I filter paper and either benzene–ethanol–water (15:4:1 v/v, upper phase) or *n*-butanol–ethanol–water (40:11:19 v/v) as solvent systems. The polyols were detected by use of the periodate–permanganate (10) and the silver nitrate – ammonia (11) spray reagents.

Alcohols were acetylated in the usual manner by reaction with excess acetic anhydride in the presence of sodium acetate (1%, based on the weight of acetic anhydride). Acetates were deacetylated by a modification of the procedure of Braun (2). The acetate (5 g.) was dissolved in anhydrous methanol (50 ml.) and treated with sodium (100 mg.) dissolved in anhydrous methanol (20 ml.) at room temperature for 30 to 48 hours. To this was added acetic acid (1 ml.) and the solution was reduced to a small volume (5 to 10 ml.) by evaporation of the solvent at 45°–50° C. To the residue was added water (5 ml.) and the solution was treated with excess ion exchange resin (Amberlite IR-120). When countercurrent distribution was employed the filtered solution was used without further treatment. In other experiments the filtered solution was evaporated to dryness and the residue was distilled *in vacuo*.

Hydrogenolysis Procedure

The same procedure as that previously described was employed. Methyl α-D-glucopyranoside, technical grade having m.p. 169°–170.5° C., $[\alpha]_D$+159.0° (in water) and 0.46% reducing value (calculated as glucose), was recrystallized three times from methanol to give a product having m.p. 170.5°–171.5° C., $[\alpha]_D$+159.4° (in water) and 0.029% reducing value. The purified product, 100 g., was hydrogenated at 240° C. and under a pressure of 170 atmospheres for 7 hours in the presence of copper chromite catalyst, 10 g., and 1000 ml. dry dioxane. The product was found to contain water, 21 g., methanol, 13 g., and yielded on evaporation of the solvent a clear colorless sirup, 65 g., n_D^{20} 1.4580. On being dried for about 3 hours on an oil pump, the residue, 55 g., had n_D^{20} 1.4630.

When the reaction time was reduced to 4 or 3 hours a product was obtained which did not differ noticeably from the above product, and paper chromatograms of the reaction product showed only traces of starting material to be present.

2-Hydroxymethyl-4,5-dihydroxytetrahydropyran

The sirup obtained from the hydrogenolyses experiments, after removal of the solvent, was fractionally distilled at 9 mm. The distillation was stopped when the temperature of the distillate reached 205° C. The residue, which constituted about one-third of the sirup, was then acetylated in the usual manner. The acetylated product was distilled *in vacuo* and yielded a viscous colorless liquid, b.p. 191.5°–193.5° C. at 12–13 mm., n_D^{20} 1.4579, d_4^{20} 1.203, $[\alpha]_D^{25}$ −1.83° (*c*, 7.04, ethanol). Calculated for $C_{12}H_{18}O_7$: C, 52.55%; H, 6.62%; acetyi, 47.1%; MR, 62.01. Found: C, 52.71%; H, 6.62%; acetyl, 47.1%; MR, 62.20; molecular weight 274.3. A product giving identical data was isolated when the residue from the fractional distillation of the acetylated hydrogenation product was distilled at 0 1–0.2 mm. pressure, and was furthermore isolated in large amounts from fractions 11 and 12 (see Table I).

The acetate was saponified in the usual manner and yielded on distillation *in vacuo* a very viscous, colorless substance, b.p. 163–167° C. (air bath) at 0.04 mm., n_D^{20} 1.5080, $[\alpha_D^{20}]$ +10.2° (*c*, 1.9 water). Calculated for $C_6H_{12}O_4$: C, 48.64%; H, 8.16%. Found: C, 48.74%; H, 8.02%. A quantitative periodate oxidation of a small amount of this compound consumed 97.8 and 100.5% of the calculated amount of oxidant (one α-glycol group per mole) in 1 and 2 hours' reaction time respectively, with no further consumption of oxidant during the next 3 hours. The free polyol formed a *p*-nitrobenzoate in good yield, which was, however, difficult to purify, m.p. 249°–250° C. Calculated for $C_{27}H_{21}N_3O_{13}$: C, 54.46%; H, 3.56%; N, 7.05%. Found: C, 54.73%; H, 3.59%; N, 6.87%.

The structure of the triol was determined as follows: 934.6 mg. (6.30 mM.) was dissolved in water (5 ml.) and to the solution was added, with shaking, periodic acid (2.96 g., 10 mM.) dissolved in water (15 ml). After 1 hour at room temperature, the solution was neutralized by the addition of 1 *N* sodium hydroxide solution (15 ml.). To this solution was added sodium borohydride (1.51 g., 22.2 mM.) dissolved in water (15 ml.). After 16 to 18 hours at room temperature the reaction mixture was neutralized with 10% hydrochloric acid solution and then evaporated at 50° C. on a rotary evaporator. To the residue was added a 4% solution of hydrochloric acid in methanol (20 ml.), and the resulting mixture was filtered. The precipitate was washed with small amounts of methanol, the filtrate and washings were combined, dried over anhydrous sodium sulphate, and then evaporated to give a viscous sirup (0.450 g., 48% of theory). This material was then distilled *in vacuo*, b.p. 140°–150° C. (air bath) at 0.03 mm. and, except for a small aliquot of about 50 mg., acetylated in the usual manner. The acetate was distilled

in vacuo, yielding 0.630 g. of the acetate of the triol-ether III, b.p. 135°–140° C. (air bath) at 0.04 mm., n_D^{20} 1.4468. Calculated for $C_{12}H_{20}O_7$: C, 52.16%; H, 7.30%; acetyl, 46.75%. Found: C, 51.99%; H, 7.32%; acetyl, 46.4%.

The triol-ether III (50 mg.) was treated with excess boron trifluoride and acetic anhydride for 3½ hours at 60° C. Methanol was added dropwise and the product was concentrated *in vacuo* to give a sirup, which was poured into an aqueous solution of sodium bicarbonate. The solution was extracted repeatedly with chloroform and the extract dried over anhydrous sodium sulphate. The solvent was evaporated *in vacuo*, the residue deacetylated, and then analyzed by paper chromatography. Spots having R_f values identical with those of ethanediol and 1,2,4-butanetriol were detected and consequently the structure III was tentatively assigned to the cleavage and reduction product.

The triol-ether III was synthesized by reaction of L-malic acid ethyl ester (IV) (3.8 g., 20 mM.) with sodium (0.46 g., 20 mM.), adding ethyl iodoacetate (V) (8.6 g., 40 mM.) and powdered potassium iodide (about 0.5 gm.) and refluxing the mixture, with stirring, in the presence of xylene (100 ml.) for 72 hours. The reaction mixture was filtered through celite filter aid, treated with sodium bicarbonate, and dried over anhydrous sodium sulphate. The solution was evaporated to dryness and the residue was distilled *in vacuo*, yielding the ester VI, b.p. 115°–120° C. (air bath) at 0.2 mm., n_D^{20} 1.4468. Calculated for $C_{12}H_{20}O_7$: saponification equivalent, 92.09. Found: saponification equivalent, 93.6. This ester was reduced with lithium aluminum hydride in the usual manner and the alcohol thus obtained was acetylated. The acetate was distilled *in vacuo*, b.p. 135°–140° C. (air bath) at 0.04 mm., n_D^{20} 1.4458. The infrared spectrum of this acetate and that of the acetate of the triol-ether III obtained from the triol I were identical.

Hexanediols

Preliminary analysis by paper chromatography of the alcohols obtained from fractions 5, 6, and 7 (see Table I) showed that these fractions were composed mainly of a compound having an R_f value of about 0.75 (benzene–ethanol–water solvent). Besides this only traces of material corresponding to 1,2-propanediol and glycerol and an unknown compound with R_f = 0.45 were detected.

An aliquot of each fraction (5.00 g.) was deacetylated and subjected to a five stage countercurrent distribution, using water (5 ml.) and chloroform (25 ml.) as solvents. The chloroform solutions were combined and evaporated to dryness. Fraction 5 yielded a crystalline product (2.50 g.), m.p. 42°–50° C. This material was recrystallized six times from petrol (b.p. 64°–66° C.), which contained a small amount of acetone, to give *meso*-3,4-hexanediol, m.p. 89.0°–89.5° C. (Young *et al.* (14) report m.p. 88.0°–88.2° C. and van Risseghem (12) m.p. 90.1°–90.2° C.). Calculated for $C_6H_{14}O_2$: C, 60.98%; H, 11.94%. Found: C, 60.81%; H, 12.01%. The 3,5-dinitrobenzoate, m.p. 190°–191° C. (reported (14) m.p. 190° C. and that of the D,L-isomer m.p. 167°–168° C.), was obtained only in fair yield and the *p*-nitrobenzoate was, therefore, prepared. This was obtained in excellent yield, crude m.p. 163°–168° C., and after four crystallizations from petrol (b.p. 64°–66° C.) containing either ethyl acetate, acetone, or 2-butanone gave a m.p. 172°–174° C., which did not change on further recrystallization. Calculated for $C_{20}H_{20}N_2O_8$: C, 57.69%; H, 4.84%; N, 6.73%. Found: C, 57.69%; H, 4.86%; N, 6.70%.

Fraction 7, when treated similarly, gave a liquid product (2.49 g.), b.p. 113°–114° C. at 13 mm., n_D^{20} 1.4412, $[\alpha]_D^{24}$ +0.2° (*c*, 4.2, water). Calculated for $C_6H_{14}O_2$: C, 60.98%; H, 11.94%. Found: C, 60.70%; H, 11.89%. Periodate oxidation of a small aliquot

yielded only propionaldehyde in about 90% of the calculated amount (as 3,4-hexanediol). Both the *p*-nitrobenzoate and the 3,5-dinitrobenzoate (obtained in poor yield) were products having a very wide melting range. Repeated recrystallizations finally yielded a *p*-nitrobenzoate of m.p. 162°–164° C. Calculated for $C_{20}H_{20}N_2O_8$: C, 57.69%, H, 4.84%; N, 6.73%. Found: C, 57.87%; H, 4.57%; N, 6.76%. It would, therefore, appear that the liquid fraction is composed mainly of DL-3,4-hexanediol.

Fraction 6 yielded a liquid product (2.60 g.) which solidified partially on cooling. Fractional crystallization from petrol (b.p. 64°–66° C.) gave a major portion (about 65%) of *meso*-3,4-hexanediol, m.p. 88.5°–89.5° C. The residue was impure DL-3,4-hexanediol.

Fraction 8 contained a large amount of an alcohol with $R_f = 0.98$ (benzene–ethanol–water solvent). This compound was obtained readily by a three stage countercurrent distribution. The infrared spectrum was indicative of a hexanediol having a primary hydroxyl group. Since the compound did not react with periodic acid to any large extent, it could be purified by treatment with an aqueous solution of periodic acid. The aqueous solution was extracted three times with chloroform and the extract dried over anhydrous sodium sulphate. The solvent was evaporated and the residual liquid distilled *in vacuo*, giving a clear, colorless liquid, b.p. 120°–125° C. (air bath) at 12 mm., n_D^{20} 1.4515, d_4^{20} 0.9818, $[\alpha]_D^{24}$ +0.3° (*c*, 4.0, water) (Glacet (6) reports for 1,4-hexanediol b.p. 127.5°–127.7° C. at 13 mm., $n_D^{16.3}$ 1.4530, $d_4^{16.3}$ 0.982, phenylurethane, m.p. 71° C., b.p. of the diacetate 120.2°–120.4° C. at 12 mm., $n_D^{16.5}$ 1.4282). Calculated for $C_6H_{14}O_2$: C, 60.98%; H, 11.94%; MR, 32.96. Found: C, 60.62%; H, 12.08%; MR, 32.45. The phenylurethane, m.p. 67°–70° C., was obtained only in poor yield, whereas the *p*-nitrobenzoate was obtained in excellent yield, m.p. 123°–124° C. after two recrystallizations (depressed by an authentic sample of di-*p*-nitrobenzoyl-1,6-hexanediol, m.p. 119°–120° C.). Calculated for $C_{20}H_{20}N_2O_8$: C, 57.69%; H, 4.84%; N, 6.73%. Found: C, 57.63%; H, 4.95%; N, 6.71%.

1,2-Propanediol, Ethanediol, and Glycerol

Analysis by paper chromatography showed that these three polyols were present in practically all but the last fraction, but large amounts were found only in fractions 2, 3, 8, 9, and 10.

Aliquots of the aqueous solutions obtained by countercurrent distribution were analyzed by the chromatographic method of Dal Nogare (3). The same procedure was employed, except that after elution of ethanediol the column was eluted further with *n*-butanol containing ethanol (20%) and finally with aqueous ethanol (50%). The identity of 1,2-propanediol, ethanediol, and glycerol was confirmed by periodate oxidation and colorimetric determination of the formaldehyde (9) and acetaldehyde (4) formed. Glycerol was confirmed by Feigl's spot test (5).

n-Hexanol

Fraction 1 was composed mainly of *n*-hexyl acetate, which yielded on deacetylation and redistillation *n*-hexanol, b.p. 155°–157° C. at 720 mm., n_D^{20} 1.487. The 3,5-dinitrobenzoate was obtained in good yield, m.p. 56°–58° C. (undepressed by an authentic sample of *n*-hexyl 3,5-dinitrobenzoate, m.p. 57.5°–58.0° C.). *n*-Hexanol was also present in small amounts in the solvent mixture obtained during the evaporation and drying of the hydrogenolysis product.

Compounds of Unknown Structure

Fractions 2, 3, and 4 contained a large amount of material which dissolved readily in the chloroform phase when countercurrent distribution was applied. The material thus obtained did not react with periodic acid to any large extent and was, therefore, purified further by treatment with an aqueous solution of periodic acid and, after extraction and drying, by distillation *in vacuo*.

Fraction 2 yielded a colorless liquid (about 75% of the fraction), b.p. 93° C. at 13 mm. Found: C, 57.67%; H, 9.68%; methoxyl, 0.65%. (Calculated for $C_7H_{14}O_3$: C, 57.51%; H, 9.65%.) This compound formed a mono-*p*-nitrobenzoate in excellent yield, m.p. 95°–96° C. Calculated for $C_{14}H_{11}NO_5$: C, 60.20%; H, 6.14%; N, 5.02%; saponification equivalent, 279.28. Found: C, 59.44%; H, 5.94%; N, 4.93%; saponification equivalent, 276. The infrared spectrum indicated that the compound was a cyclic alcohol, having possibly a 1,4-dioxane structure.

Fractions 3 and 4 yielded a colorless liquid (about 30% and 50% respectively), b.p. 90°–91° C. at 13 mm., and n_D^{20} 1.4489. Found: C, 59.11%, H, 10.16%. (Calculated for $C_5H_{10}O_2$: C, 58.80%; H, 9.87%.) The compound did not form a solid *p*-nitrobenzoate, nor was its infrared spectrum identical with that of tetrahydrofurfuryl alcohol.

ACKNOWLEDGMENT

The authors are indebted to Mr. M. Granat for technical assistance, to Mr. J. A. Baignee for the microanalyses, to Miss Agnes Epp for the infrared analysis, and to Dr. A. S. Perlin for the interest shown in this work.

REFERENCES

1. BAUER, H. F. and STUETZ, D. E. J. Am. Chem. Soc. **78**, 4097 (1956).
2. BRAUN, G. *In* Organic syntheses. Collective Vol. II. *Edited by* A. H. Blatt. John Wiley & Sons, Inc., New York. 1946. p. 122.
3. DAL NOGARE, S. Anal. Chem. **25**, 1874 (1953).
4. DESNUELLE, P. and NAUDET, M. Bull. soc. chim. France, **12**, 871 (1945).
5. FEIGL, F. and FREHDEN, O. Mikrochim. Acta, **1**, 137 (1937).
6. GLACET, C. Compt. rend. **218**, 283 (1944).
7. HOFFMAN, D. O. and WOLFROM, M. L. Anal. Chem. **19**, 225 (1947).
8. HORSLEY, L. H. Anal. Chem. **19**, 508 (1947); **21**, 831 (1949). (See also Advances in Chemistry, No. 6. Published by the American Chemical Society, Washington, D.C. 1952.)
9. LAMBERT, M. and NEISH, A. C. Can. J. Research, B, **28**, 83 (1950).
10. LEMIEUX, R. U. and BAUER, H. F. Anal. Chem. **26**, 920 (1954).
11. PARTRIDGE, S. M. Nature, **158**, 270 (1946).
12. RISSEGHEM, H. VAN. Bull soc. chim. Belg. **47**, 194 (1938).
13. RUDLOFF, E. VON. Anal. Chim. Acta. In press.
14. YOUNG, W. G., CRISTOL, S. J., and WEISS, F. T. J. Am. Chem. Soc. **65**, 1245 (1943).
15. ZARTMAN, W. H. and ADKINS, H. J. Am. Chem. Soc. **55**, 4559 (1933).

CHROMATOGRAPHY OF WASTE SULPHITE LIQUOR[1]

A. C. Shaw and M. Dignam

ABSTRACT

Waste sulphite liquor was studied by chromatographic and ion exchange techniques. A series of chromatographically homogeneous phenolic compounds, which appear to be lignin sulphonate fragments, were separated. Xylose, arabinose, galactose, and rhamnose were isolated and identified by preparation of solid derivatives.

INTRODUCTION

Polysaccharides, sugars, and lignin sulphonates form the main organic constituents of waste sulphite liquor. A more detailed knowledge of these constituents should assist in the search for uses for this waste material. Investigation of the polysaccharide fraction has already been reported (21). The present investigation deals with application of chromatographic techniques to the problem of separating and characterizing constituents of the lignin sulphonate and sugar fractions.

Adequate criteria for the purity of lignin sulphonate fractions are lacking, and with the usual fractionation procedures, e.g., dialysis and precipitation (15, 16), the boundaries between fractions are dependent upon arbitrary selection of experimental conditions. In such experiments, the lignin sulphonates behave as a group of closely related compounds whose molecular weight, physical properties, and chemical properties vary through a continuous range of values. In general, individual compounds are not isolable. However, Bailey (2) described the separation of butanol lignins into zones on paper chromatograms, and reported that chemical differences existed between lignins of different zones. Similarly a methanol lignin examined by Bland and Gatley (4) showed the presence of six constituents, but only the lowest R_f fraction had ultraviolet absorption spectra characteristic of lignin.

The sugar fraction of waste sulphite liquor has been the subject of a number of investigations. Yorston (26) separated a carbohydrate fraction from waste sulphite liquor by sorption of the sugars on a strongly basic ion exchange resin followed by elution with carbonic acid, but recoveries were less than 70%. He isolated crystalline xylose from this fraction.

The presence of glucose, mannose, xylose, galactose, and arabinose in waste sulphite liquor has been demonstrated by paper chromatographic studies (5, 23) as well as by less direct methods such as the measurement of dissociation constants of the sugar bisulphites (20, 24) and fermentation studies (20). McCarthy and co-workers isolated a lignin-free carbohydrate fraction from waste sulphite liquor (17). Constituent sugars were separated by partition chromatography and identified by partition coefficient, but were not isolated in crystalline form. Simple sugars accounted for the total reducing power of the carbohydrate fraction, but reducing substances, calculated as glucose, accounted for only 84% of the solids content of the fraction.

RESULTS AND DISCUSSION

Phenolic Materials Separated by Chromatography

Chromatograms of waste sulphite liquor exhibited a blue fluorescence under ultraviolet light from R_f 0.0 to 0.25 and in the vicinity of R_f 0.75 (*n*-butanol–water development),

[1]*Manuscript received December 11, 1956.*
Contribution from the Department of Chemistry, Ontario Research Foundation, 43 Queen's Park, Toronto 5, Ontario.

and contained areas reducing to ammoniacal silver nitrate which contained no sugars detectable by aniline phthalate. The substances responsible for these phenomena could not be isolated, free of carbohydrates, in amounts large enough for analyses, but the studies described here permitted some conclusions concerning their nature.

The chromatographic mobility of these compounds indicated solubility in organic solvents. Alcohol precipitation in combination with dialysis was therefore used to concentrate these compounds in an alcohol-soluble fraction (fraction I, Fig. 4). Although this fraction contained all of the original sugars, the data of Table I show that it had sulphur and methoxyl contents only slightly below those of the original liquor.

TABLE I

ANALYTICAL DATA OF FRACTIONS FROM DIALYSIS AND SOLVENT PRECIPITATION

	% of original solids	% methoxyl in solids	% of original methoxyl	% sulphur in solids
Original waste sulphite liquor	100	7.70	100	
Dialyzate	59.9	5.11	39.7	
Non-dialyzable material	35.2	12.18	55.6	
Fraction A	30.7	4.59	18.3	6.27
Fraction B	3.9	6.13	3.1	7.49
Fraction I	24.6	6.88	22.0	6.14
Calcium sulphate	4.8			
% recovery	99.2		99.0	

Substances which remain on the starting line of a paper chromatogram (fraction C) were removed from fraction I by column chromatography. The fraction II remaining contained the fluorescent reducing materials which move on a paper chromatogram, in mixture with the carbohydrates. These fluorescent materials and all of the methoxyl-, sulphur-, and ash-bearing compounds were removed from fraction II by anion exchange. About 53% of the solids content of the fraction, equivalent to 5.5% of the original liquor solids, was bound by the resin, and thus contained either phenolic or other acidic groups. From the data of Table II, the calculated methoxyl content of the material bound by the resin was 15.4%. The molar ratio of calcium to sulphur was 0.18 as compared with 0.50 for calcium lignin sulphonate. Thus 64% of the sulphur is not present as the calcium salt.

TABLE II

ANALYTICAL DATA OF FRACTIONS SEPARATED BY CHROMATOGRAPHY ON CELLULOSE COLUMNS

	Fraction C	Fraction II	% recovery based on fraction I
% original solids	14.2	10.4	
% of fraction I	57.7	42.3	99.8
% methoxyl in solids	4.18	8.16	85.3
% sulphur in solids	8.29	3.51	101.8
% sulphated ash in solids	20.0	2.7	98.6

Evidence for the presence of phenolic groups was provided by the observation that the fluorescent reducing materials could be detected on paper chromatograms by reaction with diazonium salts or with ferric ferricyanide. Both are general reactions of phenols

(3, 8). It is noteworthy that both reagents give color reactions with lignin (6, 9). Chromatograms of waste sulphite liquor, when sprayed with diazotized sulphanilic acid, gave a series of pink and brown spots, designated by letter R_f value and color in Table III. Essentially, the same series of spots was obtained from liquors from three different cooks.

TABLE III

LIST OF PHENOLIC MATERIALS SEPARATED BY PAPER CHROMATOGRAPHY

Spot	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
R_f (butanol–water)	0.0	0.04	0.085	0.137	0.148	0.175	0.19	0.25	0.72	0.76
R_f (butanol–ethanol–water)	0.0	0.15	0.30	0.39	0.41	0.45	0.48	0.56	0.78	0.85
Color with diazotized sulphanilic acid	Brown	Red-br.	Red	Brown	Red	Red	Red	Pink	Orange	Red

If the compounds which give rise to these spots are ionized in solution then their R_f values should be changed by change in pH. A sample of fraction I was acidified by cation exchange and its chromatographic behavior compared with that of untreated fraction I. After ion exchange treatment the R_f values of spots *c*, *d*, *e*, *f*, and *g* were almost doubled. Only spots *i* and *j* appeared to be unaffected. Spots *a*, *b*, and *h* were too ill-defined to permit definite conclusions. That the change effected by ion exchange treatment was reversible was shown by the fact that the original R_f values were obtained when acidified fraction I was chromatographed on paper impregnated with calcium carbonate (Fig. 1). Phenolic groups or other chromophores were not involved in the reversible change because no shift in the ultraviolet spectrum followed acidification of fraction I. It was concluded that spots *c*, *d*, *e*, *f*, and *g* are formed by phenolic compounds carrying acidic groups stronger than carbonic acid.

The ultraviolet absorption spectra in acidic and basic solution of materials isolated from each spot as well as the "difference spectra", according to Aulin-Erdtman (1), are shown in Fig. 3. Spot *c* was divided into subfractions based on differences in intensity of fluorescence within the spot.

The spectra of all fractions were similar. All except *i* and *j* showed maxima at 280 mμ, which shifted to 290 mμ in alkaline solution. The *i* and *j* maxima occurred at 274 mμ in acid solution. All preparations showed indications of a band at 230 mμ which in basic solution was intensified to a definite inflection or maximum and shifted to 245 mμ. According to Patterson and Hibbert (18) compounds containing the vanilloyl or veratroyl group absorb at 230, 280, and 306 mμ. The difference spectra of all preparations except *i* and *j* showed maxima at 250 and 300 mμ typical of lignin and of guaiacyl compounds which do not contain conjugated unsaturation in the side chain (1). The *i* and *j* fraction had a maximum at 245 mμ and showed higher absorption at the 230 mμ minimum and towards the longer wave lengths.

Alkaline nitrobenzene oxidation of material from spots *d* and *g* yielded vanillin, confirming the conclusions drawn from studies of ultraviolet spectra. Insufficient material was available for more complete oxidation studies.

It might be expected that calcium salts of strong acids would be separable by ion exclusion on columns of cation exchange resins in the calcium form (25). Thus carbohydrates would be distributed uniformly between water within and water surrounding the resin particles, whereas ionized calcium salts would be excluded from the zone of high

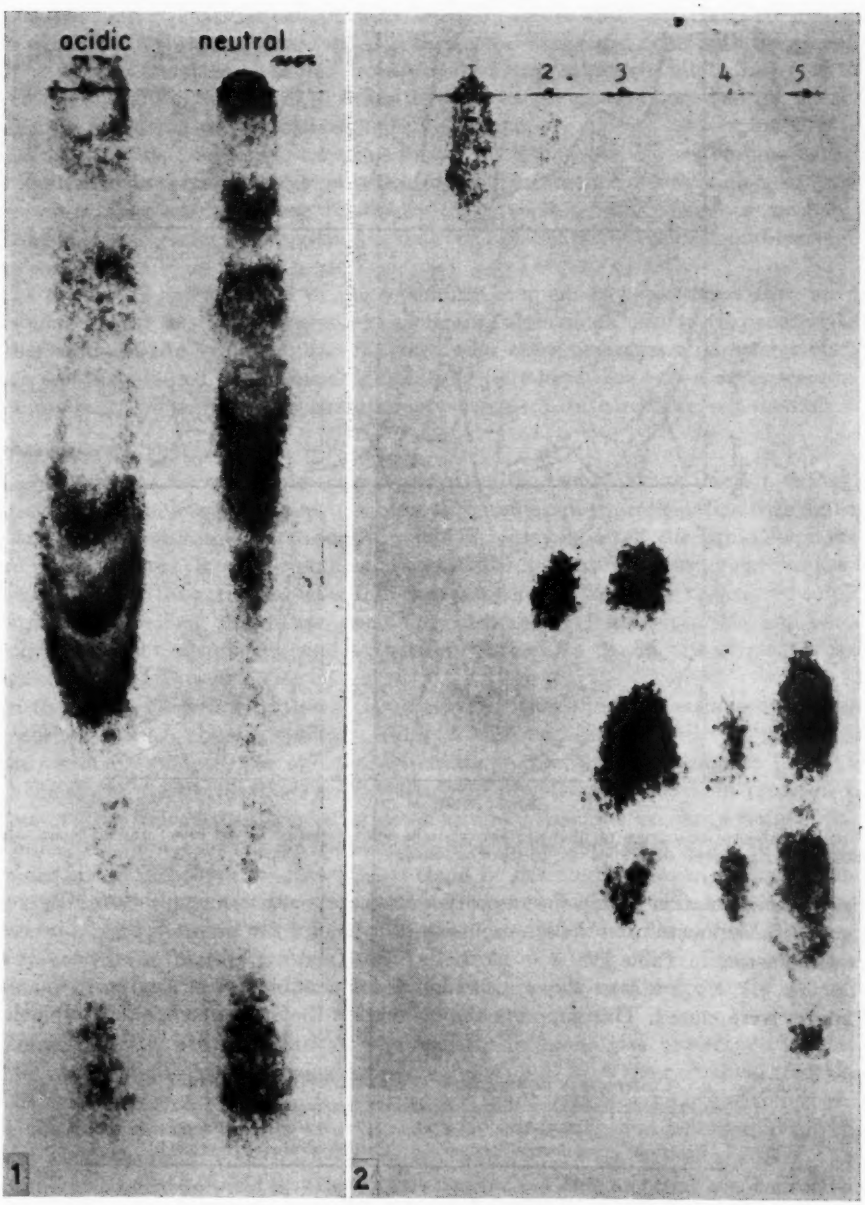


FIG. 1. Chromatograms of phenolic material in acidic and neutral solutions.
FIG. 2. Chromatograms of fractions separated by ion exclusion.

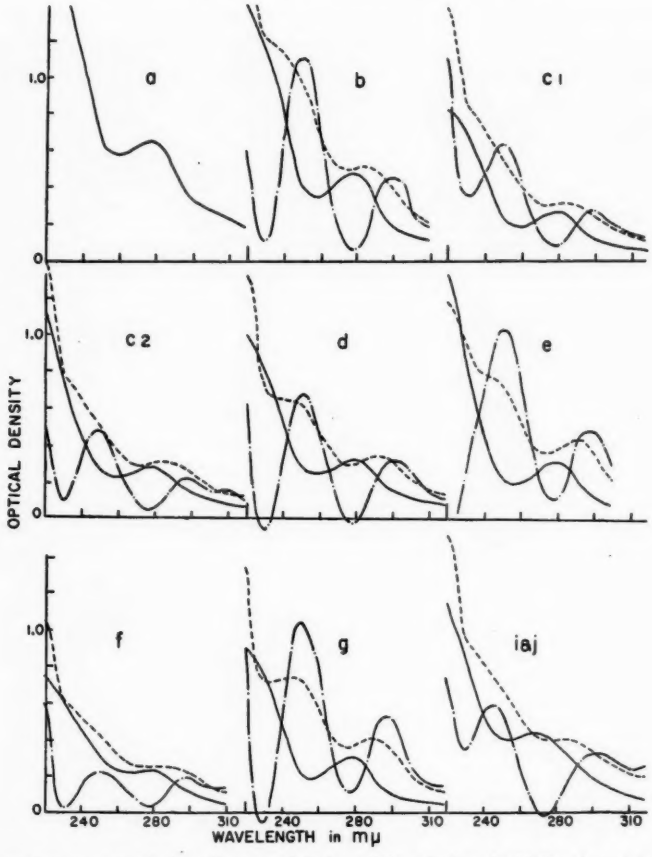


FIG. 3. Absorption curves of phenolic materials in acidic solution (solid lines) and in basic solution (dotted lines) and their difference curves (broken lines).

calcium concentration within the resin by a Donnan membrane equilibrium. Chromatograms of the effluents from the ion exclusion of fraction I are shown in Fig. 2 and results are summarized in Table IV.

Spots *a*, *b*, *c*, *e*, *g*, and possibly *f* appeared in the effluent before any significant quantities of sugars were eluted. This supports the contention that these compounds are calcium

TABLE IV

COMPOSITION OF FRACTIONS SEPARATED BY ION EXCLUSION

Fraction	Phenols in order of decreasing concn.	Carbohydrates	% of fraction I
1	*a*, *b*	Nil	20.4
2	*c*, trace *a*, *b*	Nil	21.5
3	*e*, *c*, *g*	Trace	10.8
4	*e*, *g*, *c*	Abundant	40.8
5	*d*, *g*, trace of *h*, *i*	Nil	14.3

salts of strong acids, probably sulphonic acids. Higher sulphonic acid contents and consequent higher polarities should favor lower R_f values and more complete exclusion from within the resin. It was observed that such a correlation did exist; with the exception of spot *d*, the phenols were eluted from the resin approximately in the order of their increasing R_f values. Spots *d*, *h*, *i*, *j*, and to some extent *g*, were adsorbed by the resin. More than 2 liters of water was required to elute spot *j*.

It is apparent that the *i* and *j* compounds differ from the others. Their chromatographic and ion exclusion behavior gives no indication of the presence of sulphonic acid groups. Their absorption maxima occur at slightly lower wave lengths. They are probably a mixture of simple guaiacyl compounds of the type isolated by Kvasnicka and McLaughlin from waste sulphite liquor (14).

The properties and behavior of the remaining compounds suggest that they are low molecular weight degradation products of lignin sulphonates. As starting material for further study of the lignin sulphonates, they offer the advantages of simpler molecules which can be obtained in a chromatographically pure form. Ion exclusion appears to be the most practical method of accumulating workable quantities of these materials.

Carbohydrates

Lignin sulphonates were stubbornly retained by the carbohydrate fraction through a variety of fractionation procedures (dialysis, chromatography, precipitation with amines). No anion exchange resins were found capable of removing all of the lignin sulphonates from waste sulphite liquor. Presumably most of the lignin sulphonate molecules are too large to diffuse to the reactive sites within the resin particle. However, the colored sulphur- and methoxyl-bearing impurities remaining in fraction I after dialysis and solvent precipitation were completely and irreversibly sorbed by the highly permeable resins Dowex 1-X2 and Dowex 2-X2.

In the hydroxide form, there was some evidence that these resins caused changes in the carbohydrates; column eluates contained substances not present in the original liquor, with R_f values 0.196 and 0.316, tentatively identified as mannose (R_f 0.196), and ribose (R_f 0.33) (13) from epimerization of arabinose. It has been reported that sugars may be degraded to organic acids on strongly basic ion exchange resins (19).

Difficulties did not arise when the resin was used in the carbonate form. No qualitative changes in the carbohydrate constituents could be detected by paper chromatography, and experiments with known xylose solutions showed no sorption or destruction of reducing substance by the resin. After removal of acidic materials with Dowex 1-X2 carbonate, the carbohydrate fraction III remained, in a yield of 9.08% of the liquor solids, as a colorless sirup, free of methoxyl, sulphur, and ash, which became partially crystalline on drying.

Refractionation with rejection of intermediate fractions was necessary in order to obtain chromatographically homogeneous fractions (1–5) from the carbohydrate fraction III by column chromatography. No attempt was made to obtain quantitative recoveries. The sugars were separated as white powders by triturating chromatographically pure sirups with ethanol.

Fraction 1 failed to yield any crystalline derivative. This material was subsequently shown to be a mixture of polysaccharides (21). Galactose in fraction 2 was probably contaminated with polysaccharides since on treatment with 1-phenyl-1-methylhydrazine an oil which failed to crystallize was obtained. It is improbable that the mucic acid

obtained by nitric acid oxidation of the fraction was formed from a galactose-containing polysaccharide since subsequent work showed that the highest R_f polysaccharide did not contain galactose.

Solid derivatives of arabinose and xylose were prepared from fractions 3 and 4 respectively. Rhamnose in fraction 5 was contaminated with a reducing substance which was probably not a sugar since it failed to yield a colored condensation product when heated with aniline phthalate. The mixture could not be resolved by column chromatography. Separation was carried out on paper sheets. The non-sugar reducing material was a water-soluble oil which could not be crystallized. It was not identified.

The percentage composition of the carbohydrate fraction (Table V) was calculated from gravimetric estimations of constituents separated by paper chromatography of the fraction in two different solvent systems. The greater resolving power of multiple development paper chromatography (12) as compared to column chromatography permitted quantitative separation of the simple sugars in these experiments. Simple sugars were identified by their position on the chromatogram, but, as a check, the identification of xylose was confirmed by preparation of the crystalline dibenzylidene derivative.

TABLE V

COMPOSITION OF THE CARBOHYDRATE FRACTION

Constituent	Butanol solvent		Ethyl acetate solvent	
	Distance travelled, cm.	% of fraction	Distance travelled, cm.	% of fraction
Polysaccharide	0–2	6.3	0–1	4.4
Polysaccharide	3.2	8.9	2.2	8.9
Polysaccharide	5.4	4.9	3.7	5.7
Polysaccharide	6.6	6.8	4.7	6.6
Polysaccharide	7.5	5.2	5.9	10.8
Polysaccharide and galactose	10.4–11.7	13.7	7.3	12.5
Arabinose	14.2	10.2	8.7	13.6
Xylose	16.4	31.2	11.7	29.8
?	20.6	6.5	14.2	5.6
Rhamnose	22.6	6.8	16.8	8.0
Reducing non-sugar	26.4	14.6	18.0	9.5

EXPERIMENTAL

Chromatograms were developed by descending flow. Where the object of the experiment was to isolate material, chromatograms were run on Whatman No. 3 MM paper. Whatman No. 1 was used for other chromatograms. The solvent systems used were (1) water-saturated *n*-butanol, (2) *n*-butanol:ethanol:water, 20 : 3.5 : 8, (3) ethyl acetate:acetic acid:water, 3 : 1 : 3. Phenolic materials were detected with sprays of diazotized sulphanilic acid followed by sodium carbonate (11), and with equal parts of 1% solutions of ferric chloride and potassium ferricyanide (3). Aniline phthalate and ammoniacal silver nitrate sprays were used to locate the sugars.

Preparation of Fraction I

A waste sulphite liquor* from a soft cook of mixed spruce and balsam, and commercially fermented with a strain of *Saccharomyces cerevisiae*, was submitted to batch dialysis against distilled water through cellophane membranes for 10 days. Water was changed

Kindly supplied by the Ontario Paper Co., Thorold, Ontario.

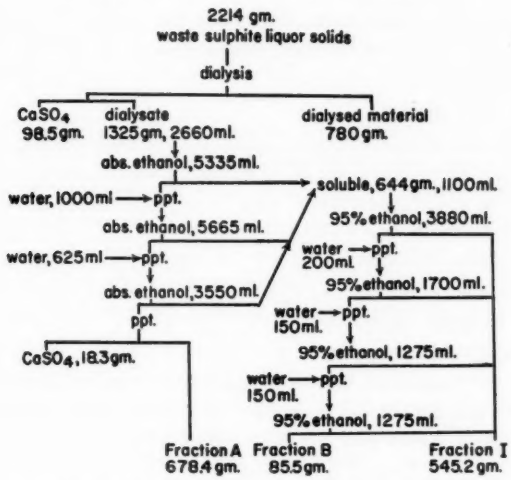


FIG. 4. Flow sheet for the preliminary fractionation of waste sulphite liquor.

on the 3rd and 6th day. The dialyzate, concentrated to about 50% solids, was fractionated by addition in a thin stream to large volumes of vigorously stirred ethanol. Details of the procedure are shown in Fig. 4. Reprecipitations were continued until sugars could no longer be detected in the precipitate by chromatography.

Preparation of Fraction II

Fraction I (10 ml. of 62.3% aqueous solution) was added to a 25 mm. column packed to a depth of 20 cm. with 20–80 mesh screenings of Whatman No. 1 filter paper and previously conditioned with water-saturated butanol. On development with water-saturated butanol, colored low R_f material moved slowly down the column in a uniform brown band with a well defined leading edge. Development was continued until the effluent was free of sugars and the brown band was within an inch of the bottom of the column. Fraction II was obtained by evaporation of the eluates. Fraction C was washed from the column with water.

Ion Exchange of Fraction II

A 2.8×28 cm. column of Dowex 1-X2, 50–100 mesh,* was treated with 200 ml. of 5% sodium carbonate solution and washed to an effluent pH of 5.5. Fraction II (100 ml. of 10% solution) was passed through the column followed by 1000 ml. of wash water. All of the sugars appeared in the first 200 ml. of effluent. The solids content of the combined effluents was 4.68 g. The resin was capable of absorbing about 0.6 g. of lignin acids per ml. of resin.

Isolation of Phenolic Material (Spots a to j)

Fraction I was applied to sheets of Whatman No. 3 paper about 14 in. long, in 0.01 ml. spots of 25% solution spaced 3/4 in. apart, and developed with water-saturated *n*-butanol. Guide strips at either edge of the sheet were cut off, and spots located with diazotized sulphanilic acid. Location of zones of resolved material was aided by examina-

**Kindly donated by the Dow Chemical Co. of Canada, Ltd.*

tion of the sheet under ultraviolet light. Zones were then cut from the sheet and eluted by a method analogous to normal chromatographic development using water as the developing solvent. A total of 0.11 g. of fraction I solids was separated in this way.

Ultraviolet Spectra of Spots a to j

The ultraviolet spectra of material from spots *a* to *j* were measured with a Beckman DU spectrophotometer in 1 cm. cells. Solid curves (Fig. 3) were measured in solution acidified with one drop of 10% sulphuric acid in 5 ml.; dotted curves were measured in solution containing 0.1 ml. of 4% sodium hydroxide in 5 ml. Because the preparations were contaminated with unknown amounts of carbohydrates, extinction coefficients could not be calculated. Values were reported as the optical densities of solutions containing the material isolated from 0.1 g. of fraction I solids in 100 ml.

Alkaline Nitrobenzene Oxidation

About 100 mg. each of spots *d* and *g* were oxidized with alkaline nitrobenzene by the method of Stone and Blundell (22). Chromatograms of the oxidation liquors sprayed with 2,4-dinitrophenylhydrazine showed the presence of vanillin.

Ion Exclusion

To a column 70 cm. in height containing 120 ml. of Dowex 50-X2, 100–200 mesh in the calcium form, 1.0 ml. of 10% solution of fraction I was added and followed with wash water at the rate of 0.5 ml./minute. Effluents were collected in 3 ml. fractions and appropriately combined in five fractions with volumes 18, 15, 12, 36, and 70 ml. respectively. Chromatograms of the effluents were prepared on Whatman No. 1 paper and developed with water-saturated butanol for 23 hours.

Isolation of Sugars

A 10% solution of fraction I, containing 31.9 g. of solids, was passed in three batches through 2.8×28 cm. columns of Dowex 1-X2 carbonate, 50–100 mesh. Column eluates plus wash water (about 2 liters) were evaporated under nitrogen to yield fraction III containing 10.8 g. of non-acidic solids.

Fraction III (9.55 g.) was applied in three batches to a 4.5×120 cm. cellulose column and developed with water saturated *n*-butanol. Eluates containing maximum concentrations of one component were combined and rechromatographed on cellulose columns. Intermediate fractions were rejected. The five fractions obtained in this way were concentrated to sirups and repeatedly triturated with absolute ethanol and evaporated in a stream of air. Fraction 1, $R_f < 0.03$, yielded 0.53 g. of white powder $[\alpha]_D$ −5° (c = 10, water) which proved to be polysaccharides. Other fractions contained simple sugars.

Galactose

Fraction 2, R_f 0.06, yielded 0.35 g. of hygroscopic white powder. Allowed to stand with 1-phenyl-1-methylhydrazine solution as described by Hirst (10), this powder (0.1 g.) yielded only an oil which failed to crystallize.

A solution of 0.20 g. of powder, 1 ml. of nitric acid (sp. gr. 1.42), and 1 ml. of water, heated at 100° for 30 minutes and cooled overnight, deposited 0.027 g. of mucic acid, m.p. 213° C.

Arabinose

The 0.26 g. of crystals which separated from fraction 3, R_f 0.100, was heated for 1 hour at 85° with an equal weight of *p*-nitrophenylhydrazine in 4 ml. of ethanol. The resulting

precipitate crystallized in yellow platelets from water and melted at 180° C. alone or in admixture with arabinose *p*-nitrophenylhydrazone.

Xylose

Two crops of xylose $[\alpha]_D$ +19.2° (c = 10, water) weighing 1.61 and 0.57 g. were obtained from fraction 4, R_f 0.125. The *p*-nitrophenylhydrazone, prepared as above, melted at 152–153° C. alone or in admixture with xylose *p*-nitrophenylhydrazone.

Rhamnose

Fraction 5, R_f 0.22, failed to deposit any crystalline material. Paper chromatography of the fraction showed the presence of a sugar, and another substance of slightly higher R_f value which did not give a color with the sugar spray reagents (aniline phthalate, *p*-anisidine hydrochloride) but which was strongly reducing to ammoniacal silver nitrate. Accordingly, 0.20 g. of fraction 5 was applied to eight 20-cm. wide sheets of Whatman No. 3 filter paper and developed with butanol, ethanol, water solvent. Zones containing the sugar were cut out, eluted with water, and the water evaporated. A small amount of crystalline material and a non-crystallizing sirup was obtained when the concentrate was triturated with absolute ethanol and evaporated. Crystals (0.05 g.) in 0.50 ml. of water were treated with 0.045 ml. of phenyl hydrazine and refrigerated overnight. The precipitate was washed with ethyl ether, and after two crystallizations from water melted at 156° C. A sample of rhamnose phenylhydrazone melted at 158° C.; the mixed melting point was 157.5° C.

The sirup (0.050 g.) was heated at 100° C. for 15 minutes with 0.050 g. of *p*-nitrophenylhydrazine in 0.6 ml. of ethanol. The precipitate which separated after 24 hours melted at 180° C., which was raised to 183.5° C. by one crystallization from ethanol. Insufficient material was available for further purification. A sample of rhamnose *p*-nitrophenylhydrazine prepared in a similar manner melted at 185° C. The mixture melted at 184° C.

Paper Chromatography of Fraction III

A total of 0.1043 g. of fraction III solids was applied to starting lines 36 cm. from the bottom of four sheets of Whatman No. 3 filter paper (23×44 cm.). Guide spots were applied at both edges of the sheets. Sheets were twice developed (12) with butanol, ethanol, water (20: 35: 8), the guide strips cut off, and sugars located with aniline phthalate and ammoniacal silver nitrate. Zones were marked, cut out, eluted with water, and the eluates freeze-dried and weighed (Table V).

In a similar experiment 0.1290 g. of fraction III solids on four sheets of Whatman No. 3 filter paper was twice developed with ethyl acetate, acetic acid, water (3: 1: 3).

Material from the xylose spot (0.0754 g.) treated with dry methanolic hydrogen chloride and benzaldehyde according to Breddy and Jones (7) gave 0.0499 g. of the dibenzylidene derivative of xylose, m.p. 209–210° C.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Drs. J. E. Milks and S. G. Reid for preliminary work leading up to this investigation, and to Dr. H. B. Marshall for many helpful suggestions. This work was supported by a research grant made to the Ontario Research Foundation by the Government of Ontario.

REFERENCES

1. AULIN-ERDTMAN, G. Chemistry & Industry, 581 (1955).
2. BAILEY, A. J. Am. Chem. Soc. **73**, 2325 (1951).
3. BARTON, G. H., EVANS, R. S., and GARDNER, J. A. F. Nature, **170**, 249 (1952).
4. BLAND, D. E. and GATLEY, F. M. Nature, **173**, 32 (1954).
5. BOGGS, L. A. Anal. Chem. **24**, 1674 (1952).
6. BRAUNS, F. E. The chemistry of lignin. Academic Press, Inc., New York, N.Y. 1952. p. 30.
7. BREDDY, L. J. and JONES, J. K. N. J. Chem. Soc. 738 (1945).
8. BROUMAND, H. and SMITH, J. H. J. Am. Chem. Soc. **74**, 1013 (1952).
9. CROSS, C. F. and BEVAN, E. J. J. Soc. Chem. Ind. (London), **12**, 104 (1893).
10. HIRST, E. L., JONES, J. K. N., and WOODS, E. A. J. Chem. Soc. 1048 (1947).
11. HOSSFELD, F. L. J. Am. Chem. Soc. **73**, 852 (1951).
12. JEANES, A., WISE, C. S., and DIMLER, R. J. Anal. Chem. **23**, 415 (1951).
13. JERMYN, M. A. and ISHERWOOD, F. A. Biochem. J. **44**, 402 (1949).
14. KVASNICKA, E. A. and MCLAUGHLIN, R. R. Can. J. Chem. **33**, 637 (1955).
15. LEOPOLD, B. Acta Chem. Scand. **6**, 64 (1952).
16. MARKHAM, A. E., PÉNISTON, Q. P., and MCCARTHY, J. L. J. Am. Chem. Soc. **71**, 3599 (1949).
17. MULVANY, P. K., AGAR, H. D., PENISTON, Q. P., and MCCARTHY, J. L. J. Am. Chem. Soc. **73**, 1255 (1951).
18. PATTERSON, R. F. and HIBBERT, H. J. J. Am. Chem. Soc. **65**, 1862 (1943).
19. PHILLIPS, J. D. and POLLARD, A. Nature, **171**, 41 (1953).
20. ROSCHIER, H. and AALTIO, E. Paper and Timber (Finland), **29**, No. 8, 189 (1950).
21. SHAW, A. C. Pulp & Paper Mag. Can. **57**, No. 1, 95 (1956).
22. STONE, J. E. and BLUNDELL, M. J. Anal. Chem. **23**, 771 (1951).
23. SUNDMAN, J. Paper and Timber (Finland), **32**, No. 9, 267 (1950).
24. SUNDMAN, J. Paper and Timber (Finland), **29**, No. 8, 113 (1947).
25. WHEATON, R. M. and BAUMAN, W. C. Ind. Eng. Chem. **45**, 228 (1953).
26. YORSTON, F. H. Pulp & Paper Mag. Can. **50**, No. 12, 111 (1949).

VEGETABLE IVORY AS A SOURCE OF A MANNAN POLYSACCHARIDE[1]

T. E. TIMELL

ABSTRACT

Native vegetable ivory has been found to contain in addition to the mannose also glucose residues together with minor amounts of galactose, arabinose, xylose, and rhamnose units. No pectin could be detected. The native material was very non-uniform and was composed of two major portions (mannans A and B) which differed considerably in their average molecular weight and polymolecularity. Alkaline extraction of the untreated product followed by purification via the copper complex offers the best means for obtaining a pure mannan polysaccharide.

The endosperm of the seed of the tagua palm (*Phytelephas macrocarpa*), commonly referred to as vegetable ivory, is the best source available for isolation of a true mannan polysaccharide. This product has been shown to be composed of 45% mannan A, 25% mannan B, and 7.5% cellulose, the remainder constituting a material removed by treatment with sodium chlorite (7, 9). The nature of the latter component is unknown. It has been tentatively suggested that it is a pectic material (9) but it has also been referred to as a lignin (3). Mannans A and B have recently (3) been found to differ mainly in molecular size, exhibiting degrees of polymerization of 10 and 40, respectively. Both are apparently composed of 1,4-linked mannopyranose units, although a few 1,6-linkages are presumably also present.

In connection with a study of a glucomannan from wood (14) it became necessary to prepare a pure mannan polysaccharide from vegetable ivory. It was soon noticed that the technique usually adopted for this purpose (3, 9) was not the most suitable one and that the general composition of vegetable ivory needed further investigation.

EXPERIMENTAL

Material

Vegetable ivory nuts were polished with quartz sand in a ball mill and reduced to thin shavings on a lathe, after which they were extracted with ethanol–benzene (1:2 v/v). A part of the shavings was converted to a 40–60 mesh meal in a Wiley mill. This material, however, proved less satisfactory than the shavings.

Methods

Analytical Procedures

The ivory nut shavings were converted to the corresponding mixture of sugars by the method of Saeman and co-workers (11). The monosaccharides were separated on Whatman No. 1 filter papers using the ethyl acetate – acetic acid – water (9:2:2) and the *n*-butanol–pyridine–water (10:3:3) solvent systems. After elution from the paper with water, the sugars were determined in triplicate analyses by the spectrophotometric *o*-aminodiphenyl method of Timell, Glaudemans, and Currie (12). A portion of the hydrolyzate was treated with baker's yeast for elimination of mannose and glucose. After removal of the yeast by filtration through Celite, the relative amounts of sugars were

[1]*Manuscript received October 24, 1956.*
Contribution from the Department of Chemistry, McGill University, and the Wood Chemistry Division, Pulp and Paper Research Institute of Canada, Montreal, Que.

estimated as above. Nitrogen analyses were carried out by a semimicro Kjeldahl procedure (13). Standard methods indicated the absence of Klason lignin and methoxyl groups in the native product (9).

Reduced viscosities were measured in 1 *M* cupriethylenediamine and extrapolated to zero concentration with Martin's equation (10). The constant *K* in the latter was determined as 0.16. Nitrate viscosities were estimated in acetone, a value of 0.20 being found for *K* in this case. Both measurements were performed with Cannon-Fenske viscometers. The relationship D.P. = 100 $[\eta]$ was used for converting the intrinsic viscosities of the nitrates to degrees of polymerization.

Identification of Monosaccharides

Glucose, xylose, and rhamnose were identified chromatographically. The sugar mixture obtained after fermentation of the hydrolyzate was separated on large sheets of Whatman No. 1 filter papers with the butanol solvent system. Galactose was identified as the N-methyl N-phenylhydrazone (4), m.p. and mixed m.p. 190° C. Arabinose was characterized by the benzoylhydrazone (4), m.p. and mixed m.p. 190° C. The xylose component failed to give a crystalline dibenzylidene dimethyl acetal derivative. Mannose was identified in the original hydrolyzate as the phenylhydrazone, m.p. and mixed m.p. 198° C.

Treatment with Sodium Chlorite

Ivory nut shavings (94.7 g.) were treated at 70° C. with 4000 ml. of an aqueous solution containing 30 g. of sodium chlorite and 20 ml. of glacial acetic acid, fresh amounts of the latter two reagents being added every hour (16). After a total time of eight hours, the remaining material was isolated by filtration, washed with distilled water, and dried to give 78.0 g., corresponding to a yield of 79.2%.

Extraction with Ammonium Oxalate

Ivory nut shavings (9.4 g.) were treated at 70° C. for 3 hours with 600 ml. of a 0.5% solution of ammonium oxalate in water. Unextracted material amounted to 7.55 g., corresponding to a yield of 80%. Only a small portion of the extract could be recovered by addition of acidified ethanol.

Nitration and Fractionation

Ivory nut shavings (19.0 g.) were nitrated at +17° C. for 4 hours with 700 ml. of a mixture containing nitric acid, phosphoric acid, and phosphorus pentoxide in the ratio 64:26:10 (1). After removal of the spent acids by filtration, the product was washed with distilled water for two days and finally dried *in vacuo* at 60° C., giving 28.1 g. of a nitrate containing 14.1% nitrogen, corresponding to a yield of 80.5%. Other materials were nitrated in a similar way.

Ten grams of the above product was dissolved in 1400 ml. of reagent grade acetone containing 8% of water. Acetone was slowly removed with a current of air until the formation of a precipitate was indicated by the appearance of a slight opalescence. After equilibration for 10–15 minutes, the precipitate was removed by centrifuging and the supernatant solution was subjected to further fractionation. The fraction was isolated by evaporation of the solvents. In this way a total number of 45 fractions was obtained, most of them ranging in weight between 150 and 250 mg. Intrinsic viscosities were determined for all of them and a few were also denitrated with 20% aqueous ammonium hydrosulphide for subsequent sugar analysis.

RESULTS AND DISCUSSION

The absence of any methoxyl groups as well as the failure to isolate any Klason lignin from vegetable ivory on treatment with strong sulphuric acid suggested that no lignin was present. Uronic acids could not be detected in the sugar mixture obtained after hydrolysis, thus indicating that pectin was probably also absent. In addition to mannose, glucose, galactose, arabinose, xylose, and rhamnose were identified. All sugars were separated on paper chromatograms and quantitatively determined, mannose and glucose having been removed from one hydrolyzate by fermentation. The results are given in Table I. The hydrolyzate of native vegetable ivory apparently contains noticeable

TABLE I

COMPOSITION OF HYDROLYZATE FROM IVORY NUT BEFORE AND AFTER FERMENTATION

Component	Per cent	
	Original	Fermented
Mannose	86.00	—
Glucose	10.56	—
Galactose	2.04	59.6
Arabinose	1.07	31.0
Xylose	0.33	9.4
Rhamnose	Trace	—

quantities of glucose besides the major mannose constituent, and in addition there are also present minor amounts of galactose, arabinose, xylose, and rhamnose. Fifty years ago pentoses and methylpentoses were reported to be present in vegetable ivory (5) but this observation was not corroborated by subsequent experience (15). Pentosans are sometimes detected together with the main galactomannan constituent of the endosperm from legumes (2) but are more often absent (2, 8). Xylose units have been found to accompany the mannan portion of a seaweed, *Porphyra umbilicalis* (6).

Although the absence of any uronic acid groups as well as the complete solubility of ivory nut shavings in cupriethylenediamine, which does not dissolve pectin, suggested the absence of any pectic material, additional evidence was deemed desirable. A known amount of material was converted to the corresponding sugar mixture, the total monosaccharide content of which was determined. In a parallel experiment a mixture containing the same relative amounts of sugars as the ivory nut was also subjected to treatment with sulphuric acid and subsequently analyzed. Comparison between the results showed that 98.8% of the ivory nut consisted of reducing sugar units. A similar experiment, where ribose was added as a reference sugar, gave a value of 98.7%. The only constituents in vegetable ivory are accordingly the sugar residues indicated in Table I and neither lignin nor pectin seem to be present.

Treatment with acidified sodium chlorite and with dilute aqueous ammonium oxalate corroborated the above results. The latter reagent, which is a good solvent for pectin, removed almost the same amount of material as did the chlorite treatment, as can be seen from Table II. However, in both cases the composition of the material remaining after completed extraction was nearly the same as that of the original product.

Mannan has hitherto been prepared from vegetable ivory by alkaline extraction of the material after a prior treatment with chlorite (3, 9). Yield and composition of the

TABLE II

COMPOSITION OF UNTREATED AND MODIFIED IVORY NUT PRODUCTS

Material	Yield, %	$[\eta]$*	Per cent			
			Galactan	Glucan	Mannan	Pentosan
Untreated	—	1.52	2.0	10.6	86.0	1.4
Treated with $NaClO_2$	79.2	0.27	1.4	10.0	88.2	0.4
Treated with $(NH_4)_2C_2O_4$	80.0	—	1.4	12.7	86.2	—

**dl./g. in 1 M cuprietylenediamine.*

products obtained by alkaline extraction of untreated ivory nut shavings are given in Table III. The corresponding values noted with a material previously digested with sodium chlorite are also presented in Table III. The composition of the various mannans

TABLE III

EXTRACTION OF UNTREATED AND TREATED IVORY NUT SHAVINGS WITH AQUEOUS SODIUM HYDROXIDE

Alkali, %	Extract, %	Per cent		
		Galactan	Glucan	Mannan
	Untreated			
5	46.7	1.5	1.4	97.1
10	56.4	0.7	2.2	97.1
17.5	62.8	1.9	5.3	92.8
17.5+4% H_3BO_3	76.6	2.2	6.8	91.0
	Previously treated with sodium chlorite			
5	54.5	1.6	1.5	96.9
10	87.2	1.2	2.6	96.2
17.5	91.8	0.8	1.4	97.8
17.5+4% H_3BO_3	91.8	2.3	1.5	96.2

is evidently almost the same throughout. The higher yield obtained with the product treated with chlorite is undoubtedly due to the much lower average chain-length of the latter (cf. Table II). The best method for isolating a relatively unchanged mannan polysaccharide from vegetable ivory is therefore to extract the untreated material with 5–10% aqueous sodium hydroxide in an atmosphere of nitrogen. Two or three purifications of the crude mannan via its copper complex (3) result in a product containing only traces of anhydrogalactose and glucose units.

When untreated ivory nut shavings were nitrated with a non-degrading acid mixture and the material was dissolved in acetone and subsequently precipitated into water, only half of the material was recovered, as is seen in Table IV. The composition was

TABLE IV

NITRATION OF IVORY NUT SHAVINGS

Material	Yield, %	$[\eta]$*	Per cent		
			Galactan	Glucan	Mannan
Untreated	80.5	3.09	2.2	14.9	82.9
Untreated†	47.7	5.74	1.4	23.7	74.9
Treated with $NaClO_2$	66.4	0.93	—	—	—

**dl./g. in acetone.*

†Fraction precipitated into water from acetone solution.

also different from that of a nitrate that had not been reprecipitated. The higher glucan content in the former case indicates that this product contained relatively more cellulose because the low-molecular-weight mannan had not been recovered completely. This conclusion is also supported by the higher intrinsic viscosity of the precipitated portion.

The nitrate obtained in the higher yield was subjected to a fractional precipitation procedure, 45 fractions being isolated in a one-stage experiment using the system acetone–water. Intrinsic viscosity of the fractions was determined in acetone, and seven of them were also denitrated, hydrolyzed, and analyzed for sugar constituents. The weight frequency distribution thus obtained is presented in Fig. 1. The material apparently con-

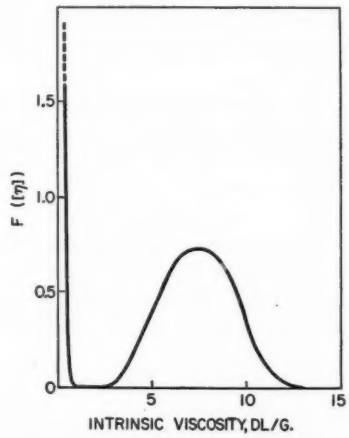


FIG. 1. Weight frequency distribution of nitrated, native vegetable ivory.

sisted of two distinct portions. One of them, comprising 60% of the total, contained oligosaccharides with 5–7 sugar units whereas the remainder was polymolecular and showed degrees of polymerization extending from 300 up to 1200. The data presented in Table V indicate that the first few fractions contained more anhydrogalactose and glucose units than the following ones. The main part of the material forming the first maximum in the distribution curve consisted of slightly more than 90% of mannose residues. The greater part of the chains within the second peak contained somewhat more anhydroglucose units. The last fraction was a glucan, accompanied by some anhydromannose units. This material was probably a cellulose, contaminated by occluded mannan.

It is evident that native ivory nut is a very non-uniform material as regards both chemical composition and chain-length distribution. Extraction of untreated ivory nuts with alkali leaves only a negligible residue undissolved. Precipitation of the extract into acidified ethanol gives, however, a yield of only 60–70%, the low-molecular-weight portion remaining in solution. The chemical constitution of the mannan forming the high-molecular-weight part has been elucidated, albeit only partially (3). Information concerning the major, low-molecular-weight moiety is still lacking. Considering the composition of other endosperms, it does not appear impossible that both glucomannans and galactomannans might be present besides the mannan component.

TABLE V

COMPOSITION OF SOME OF THE FRACTIONS OBTAINED ON FRACTIONATION OF NITRATED IVORY NUT SHAVINGS

Fraction No.	Weight, %	$[\eta]$*	Per cent			
			Galactan	Glucan	Mannan	Pentosan
1–3	2.4	0.05	5.3	37.2	54.0	3.5
7–8	55.7†	0.05	3.0	6.7	90.3	—
12		0.06	1.7	4.5	93.8	—
26–27		4.10	—	13.6	86.4	—
32	40.0§	5.64	3.0	7.2	89.8	—
36–38		8.41	1.7	13.6	84.7	—
45	1.9	11.75	—	93.7	6.3	—

*dl./g. in acetone.
†Total percentage of fractions 4–25.
§Total percentage of fractions 26–44.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Mr. D. Stoltzenburg who carried out a part of the experimental work.

REFERENCES

1. ALEXANDER, W. J. and MITCHELL, R. L. Anal. Chem. **21**, 1497 (1949).
2. ANDERSON, E. Ind. Eng. Chem. **41**, 2887 (1949).
3. ASPINALL, G. O., HIRST, E. L., PERCIVAL, E. G. V., and WILLIAMSON, I. R. J. Chem. Soc. 3184 (1953).
4. HIRST, E. L., JONES, J. K. N., and WOODS, E. A. J. Chem. Soc. 1048 (1947).
5. IVANOV, S. J. Landwirtsch. **56**, 217 (1908); Chem. Abstr. **3**, 427 (1909).
6. JONES, J. K. N. J. Chem. Soc. 3292 (1950).
7. KLAGES, F. Ann. **509**, 159 (1934); **512**, 185 (1934).
8. LEW, B. and GORTNER, R. A. Arch. Biochem. **1**, 325 (1943).
9. LÜDTKE, M. Ann. **456**, 201 (1927).
10. MARTIN, A. F. Tappi, **34**, 363 (1951).
11. SAEMAN, J. F., MOORE, W. E., MITCHELL, R. L., and MILLETT, M. A. Tappi, **37**, 336 (1954).
12. TIMELL, T. E., GLAUDEMANS, C. P. J., and CURRIE, A. L. Anal. Chem. **28**, 1916 (1956).
13. TIMELL, T. E. and PURVES, C. B. Svensk Papperstidn. **54**, 303 (1951).
14. TIMELL, T. E. and TYMINSKI, A. Unpublished results.
15. WHISTLER, R. L. and SMART, C. L. Polysaccharide chemistry. Academic Press, Inc., New York. 1953. p. 152.
16. WISE, L. E., MURPHY, M., and D'ADDIECO, A. A. Paper Trade J. **122**, No. 2, 35 (1946).

INTERNAL ROTATION

IX. THE INFRARED AND RAMAN SPECTRA OF LIQUID NORMAL ALKYL BROMIDES[1]

T. YOSHINO[2] AND H. J. BERNSTEIN

ABSTRACT

The ratio of the Raman intensity of the trans to gauche bands and also the optical density ratio, for *n*-alkyl bromides, has been examined in liquid, dilute solution, and vapor (for *n*-propyl bromide) and the temperature dependence of the Raman intensity ratio obtained over the range −80° to +80° C. for the liquids. Both Raman and infrared ratios become constant for the higher members of the series but are measurably different for *n*-butyl and *n*-propyl bromide. The results are consistent with a very small difference in energy between gauche and trans forms in liquid, solution, or vapor, and can be interpreted semiquantitatively by assuming appropriate trans–gauche energy differences in the carbon chain.

The normal alkyl bromides may have various configurations due to the trans–gauche rotational isomerism about each C—C bond except the terminal one ending in a CH_3 group. For simplicity hereafter we shall call the molecule which has a trans or gauche configuration with respect to the C—C bond adjacent to the C—Br bond a *T* or *G* type molecule respectively.

The infrared and Raman spectra of propyl bromide have been investigated recently and the bands at 645 and 565 cm.$^{-1}$ were assigned to the C—Br stretching vibration of the trans (*T*) and gauche (*G*) forms respectively (2, 3, 5). The *T* and *G* type isomers for the *n*-alkyl bromide are shown schematically in Fig. 1. Here R is a normal alkyl

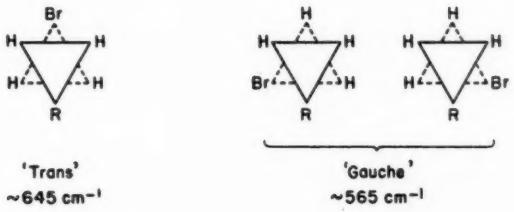


FIG. 1. Rotational isomers of *n*-alkyl bromide.

group or CH_3 for *n*-propyl bromide. There may also be trans and gauche configurations due to rotations about the various C—C bonds in the alkyl group. In these chains three different configurations are posssible with respect to rotation about each C—C bond. We denote by *t* the trans configuration, by g^+ the gauche configuration obtained by a clockwise rotation, and by g^- the gauche configuration obtained by a counterclockwise rotation. A particular isomer of a *n*-alkyl bromide can then be expressed by a combination of symbols such as

$$G^+tg^-g^-tg^+.$$

Since in all the *n*-alkyl bromides investigated the CBr bands due to the *T* and *G* type configurations remain approximately at 645 cm.$^{-1}$ and 565 cm.$^{-1}$ and no other fundamental

[1]*Manuscript received January 7, 1957.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 4250.
[2]*National Research Laboratories Postdoctorate Research Fellow 1955–.*

mode is expected in these regions, we may regard the band at 645 cm.$^{-1}$ as a superposition of C—Br stretching bands from all T type configurations and that at 565 cm.$^{-1}$ as due to the CBr bands from all G type configurations.

Let the intensities of the Raman bands belonging to the ith configuration of a T type isomer and the jth configuration of a G type isomer be denoted by I_T^i and I_G^j respectively. The observed intensity ratio is given then by

$$[1] \qquad \frac{I_{645}}{I_{565}} = \frac{I_T}{I_G} = \frac{\sum_i I_T^i \exp(-\Delta F_T^i/RT)}{\sum_j I_G^j \exp(-\Delta F_G^j/RT)}.$$

Here ΔF_T^i and ΔF_G^j are the free energies of the ith trans configuration and the jth gauche configuration referred to the standard configuration in which all orientations are trans (i.e., the completely staggered or zigzag configuration, $Tttt\ldots$); R and T have their usual meaning and the summation is over all i trans and j gauche configurations. If I_T^i and I_G^j are assumed constant, equation [1] becomes

$$[2] \qquad \frac{I_T}{I_G} = \frac{I_T^i \sum_i \exp(-\Delta F_T^i/RT)}{I_G^j \sum_j \exp(-\Delta F_G^j/RT)}.$$

If the infrared optical density D is used, equation [2] may be written for the ratio of the density of the 645 cm.$^{-1}$ band to the 565 cm.$^{-1}$ band as

$$[3] \qquad \frac{D_{645}}{D_{565}} = \frac{D_T}{D_G} = \frac{D_T^i \sum_i \exp(-\Delta F_T^i/RT)}{D_G^j \sum_j \exp(-\Delta F_G^j/RT)}.$$

I_T/I_G and D_T/D_G give relative values of the T/G mole ratio in the series of homologous compounds.

EXPERIMENTAL

All compounds were Eastman Kodak chemicals once distilled. The infrared spectra were obtained with a Perkin Elmer Model 12C spectrometer with KBr optics (slits ~4 cm.$^{-1}$). The same cell (1 mm.) was used throughout and the concentration of the n-alkyl bromide in the solvent (cyclohexane) was the same (0.2 M) for all molecules. In a separate experiment on n-butyl bromide it was determined that the ratio of the optical density of the trans band to that of the gauche band was independent of concentration. Areas under the band contours ±20 cm.$^{-1}$ about the peak were measured to obtain optical densities. The results are shown in Table I. The error in the ratio D_T/D_G is estimated to be ±10%. The infrared spectra of gaseous n-propyl bromide at 22° and 206° C. were obtained in a heated cell previously described (1) and are shown in Fig. 2.

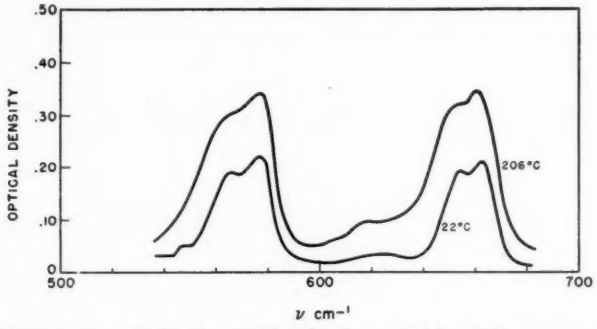


FIG. 2. Infrared spectra of the T and G bands of n-propyl bromide at two temperatures.

TABLE I

THE INFRARED DENSITIES OF THE C—Br STRETCHING BANDS OF THE *n*-ALKYL BROMIDES

	Wave number				
	Trans	Gauche	D_T	D_G	D_T/D_G
n-Propyl	651.5	566.9	79	93	0.85
n-Butyl	647.6	567.1	88	94	0.93
n-Amyl	646.3	566.6	99	90	1.10
n-Hexyl	648.4	566.7	92	92	1.00
n-Heptyl	649.6	567.0	98	98	1.00
n-Octyl	649.2	566.9	98	94	1.03
n-Nonyl	649.2	566.8	98	95	1.03
n-Decyl	649.9	567.0	92	91	1.01
n-Undecyl	649.3	566.9	92	88	1.05
i-Amyl	651.0	568.3	122	82	1.49

The Raman spectra were obtained with a White Grating Raman spectrometer with photoelectric recording (9) (slits ~7 cm.$^{-1}$). Depolarization ratios were measured by the method of Edsall and Wilson (4). The exciting line is Hg 4358 and a saturated solution of $NaNO_2$ was used as filter. The results are shown in Table II. In this Table the inte-

TABLE II

THE RAMAN INTENSITIES OF THE C—Br STRETCHING BANDS OF THE *n*-ALKYL BROMIDES

	$\Delta\nu$, cm.$^{-1}$					Observed dep. ratios		$\frac{I_T}{I_G}\frac{1+\rho_G}{1+\rho_T}$
	Trans	Gauche	I_T	I_G	I_T/I_G	ρ_T	ρ_G	
n-Propyl	647	563	.137	.332	$.41_4$	.60	.46	.38
	646	561	.120	.272	$.44_1$	.55	.45	.41
n-Butyl	641	559	.164	.296	$.55_2$	.59	.48	.51
n-Amyl	641	559	.123	.182	.676	.58	.47	.63
n-Hexyl	646	562	.114	.170	.671	.53	.44	.63
	646	562	.058	.079	.732	.53	.44	.69
n-Heptyl	643	561	.119	.161	.74	.58	.48	.69
n-Octyl	643	561	.074	.102	.728	.55	.45	.68
n-Decyl	646	564	.044	.056	.786	.58	.49	.74
i-Amyl	640	557	.139	.152	.913	.62	.47	.83

grated intensities for the trans and gauche bands, I_T and I_G, are given in arbitrary units and the ratio I_T/I_G has been corrected for convergence error and given in the last column. Two runs are shown for *n*-propyl and *n*-hexyl bromide to indicate the reliability of the measured intensity ratios. The error in the ratio I_T/I_G is estimated to be about $\pm 10\%$.

In order to investigate the temperature dependence of the ratio I_T/I_G the Raman tube was enclosed in an outer jacket through which thermostatically controlled cold or hot liquid could be circulated. The values of I_T/I_G obtained at different temperatures for some of the *n*-alkyl bromides are shown in Table III. Since the depolarization ratios for the 645 and 565 cm.$^{-1}$ bands of the various molecules are constant within the experimental error, the ratio I_T/I_G will be used in discussing the results.

In Fig. 3 the average value of the ratio I_T/I_G from Table III has been plotted against the number of *C* atoms in the molecule. In the case of *n*-hexyl and *n*-decyl bromide the values from Table II have been used.

TABLE III

I_T/I_G AS A FUNCTION OF TEMPERATURE

T, ° C.	Propyl	Butyl	Amyl	Heptyl	Octyl
80		.57	.71	.74	.79
60	.45				
50			.74	.74	.73
40		.55			
30	.44				
21		.52	.69	.74	.73
0	.47	.52			
−10				.68	.77
−20			.66		
−30	.45				
−50			.57	.78	.80
−80			.69		
Average	.45	.54	.70	.74	.76

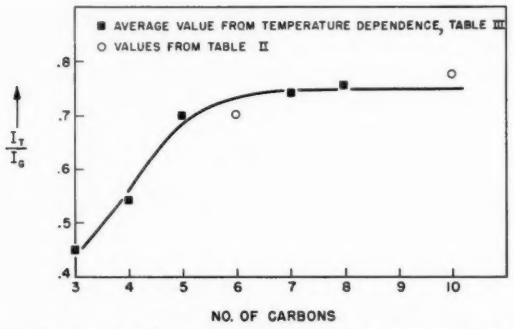


FIG. 3. The dependence of I_T/I_G on the number of carbon atoms in *n*-alkyl bromide.

DISCUSSION

From Table III it is evident that there is no temperature dependence for the liquids within the experimental error. The average energy difference* between trans and gauche forms is then very nearly zero in the liquid.

The dielectric constant of the lower members of the alkyl bromides is higher than that of the higher members (8). One might expect therefore that the reaction field of lower members would change by dilution in non-polar solvents. This gives no appreciable changes of trans–gauche energy difference nor trans–gauche mole ratio, since the dipole moments of gauche and trans forms are expected to be very nearly the same (6).

The ratio I_T/I_G for *n*-propyl bromide was measured also for cyclohexane solutions of concentrations between 1 and 0.1 mole ratio and it was found that dilution had no effect on the value of I_T/I_G. We may consider therefore that the mole ratios of the alkyl bromides are the same for liquid and solution.

The mole ratio of *n*-alkyl bromide given by D_T/D_G is very nearly the same for vapor and solution and again this is to be expected because of the nearly equal dipole moments of the trans and gauche forms (6). It is evident from Fig. 2 that the energy difference between trans and gauche *n*-propyl bromide in the vapor is also practically zero. We may conclude therefore that the energy difference in the gas phase for the *n*-alkyl bromide is nearly zero.

**The definition of the average energy difference appears later in equation* [5].

The ratio D_T/D_G shows the same trend as in Fig. 3 for the Raman intensity ratio but the change is not as great from *n*-propyl bromide through to the higher members of the series.

We shall try to interpret the results of Tables I and II and Fig. 3, by taking into account the fact that isomers of the *g* type (where rotations about C—C bonds in the alkyl chain are involved) may have different populations because of different relative stabilities. For example, in a molecule of the type G^+g^- the distance between the terminal carbon atom and the bromine atom is nearly the same as that between the first and fifth carbon atoms of the cyclohexane molecule. The repulsive forces between the terminal methyl group and bromine atom make this form unstable compared to the form G^+g^+. Furthermore it is reasonable to assume that the configuration denoted by $G^+g^-g^+$ does not actually exist, since here the distance between the terminal carbon and the bromine atom is about the same as that between the 1 and 6 positions of cyclohexane. Such highly unstable forms are more frequently encountered in the *G* type configurations than in the *T* type.

The energy differences between *t* and *g* forms contribute to ΔF_T^i and ΔF_G^j. If the large contributions to ΔF_T^i and ΔF_G^j due to configurations described above were neglected and if ΔF_T^i and ΔF_G^j could be estimated by simply adding *t*–*g* energy differences, then the *T*/*G* mole ratio

$$r = \frac{\sum_i \exp(-\Delta F_T^i/RT)}{\sum_j \exp(-\Delta F_G^j/RT)} \qquad [4]$$

would be constant for all the alkyl bromides. This is so because the abundance ratio of any of the *T* type configurations to the configuration which is the same except for the replacement of *T* by *G* is equal to twice the ratio (r) for *n*-propyl bromide. As seen from Fig. 3, r is not constant so that it is necessary to introduce energy differences of *g* forms, corresponding to situations described above for G^+g^-, G^+g^+, etc., for example. To interpret the experimental results the ratio r was calculated on the basis of the following assumptions:

(i) If a molecule has n pairs of non-bonded carbon atoms separated by the distance corresponding to the 1 and 4 positions of the cyclohexane ring, the configuration is weighted by $(\frac{1}{4})^n$. The factor of $\frac{1}{4}$ corresponds approximately to $\Delta E = +0.8$ kcal., which is the experimental value found for the trans–gauche energy difference in *n*-butane (7).

(ii) If a molecule has n pairs of non-bonded atoms in the skeleton separated by the distance nearly corresponding to the 1 and 5 positions of the cyclohexane ring, the configuration is weighted by $(\frac{1}{5})^n$.

(iii) If a molecule has a pair of non-bonded atoms in the skeleton separated by the distance nearly corresponding to the 1 and 6 positions of the cyclohexane ring, the configuration is neglected.

In (i) and (ii) ΔF is assumed to be equal to ΔE.

The calculated *T*/*G* mole ratios (r) for the *n*-alkyl bromides and isoamyl bromide are shown in Table IV referred to *n*-propyl bromide. The calculated values are compared with those observed for I_T/I_G and D_T/D_G normalized so that the constant I_T/I_G ratio of 0.75 (from Fig. 3) is made equal to 1.17 and the constant D_T/D_G ratio of 1.03 (see Table II) is also made equal to 1.17. Since the experimental error in the ratio I_T/I_G and D_T/D_G is $\sim\pm 10\%$, the relative ratios may be in error by as much as $\pm 20\%$ although this seems an overestimate.

TABLE IV

RELATIVE MOLE RATIOS OF TRANS TO GAUCHE

Molecule	Trans/gauche, calc.	Observed	
		I_T/I_G	D_T/D_G
n-Propyl	1	0.7	1
n-Butyl	1.17	0.9	1.1
n-Amyl	1.16	1.1	1.3
n-Hexyl	1.17	1.2	1.2
n-Heptyl	1.17	1.2	1.2
n-Octyl	1.17	1.2	1.2
n-Nonyl	1.17		1.2
n-Decyl	1.17	1.2	1.2
n-Undecyl	1.17		1.2
n-Osoamyl	1.95	1.5	1.8

We discuss the temperature dependence of the mole ratio by differentiation of [4] with respect to T and obtain

$$[5] \qquad \frac{T}{r}\frac{\partial r}{\partial T} = \frac{\sum_i -x_i \exp(-x_i)}{\sum_i \exp(-x_i)} - \frac{\sum_j -x_j \exp(-x_j)}{\sum_j \exp(-x_j)} = \frac{\epsilon}{RT},$$

where $x_i = \Delta F_T^i/RT$ and $x_j = \Delta F_G^j/RT$. The average energy difference ϵ as defined by [5] has been calculated at 300° K. for some alkyl bromides on the same assumptions as for the calculation of r and referred to *n*-propyl bromide as zero. It was found that ϵ for *n*-butyl, *n*-amyl, *n*-hexyl, and isoamyl bromides is -64, $+70$, $+78$, and -97 cal./mole respectively.

Since $\partial r/\partial T$ is equal to $r\epsilon/(RT^2)$, it is evident that the change in r with temperature is very small. Even in the case of isoamyl bromide with the largest r and ϵ the calculated $\partial r/\partial T$ is too small to give a measurable temperature dependence of r. This was checked experimentally from 0° to 80° C. and the change in I_T/I_G was found to be within the range of the experimental error.

The experimental results have been adequately explained on the assumption that the Raman and infrared intensities of the C—Br band for different kinds of molecules of the T or G type configuration and for different trans and gauche configurations of the same molecule have remained constant. If the observed ratio I_T/I_G for *n*-propyl bromide is really different from that calculated on the above consideration (viz. 0.7 ± 0.2 compared with 1) it seems that the Raman intensities of the T and G bands here are not the same as for the other bromides.

ACKNOWLEDGMENT

The Raman spectra of the liquids were obtained and measured by Dr. J. C. Evans while a Postdoctorate Research Fellow 1954 and some of the infrared spectra were obtained by Dr. K. Ito while a Postdoctorate Research Fellow 1954.

REFERENCES

1. BERNSTEIN, H. J. J. Chem. Phys. **18**, 897 (1950).
2. BROWN, J. K. and SHEPPARD, N. Discussions Faraday Soc. **9**, 144 (1950).
3. BROWN, J. K. and SHEPPARD, N. Trans. Faraday Soc. **50**, 1164 (1954).
4. EDSALL, J. T. and WILSON, E. B., JR. J. Chem. Phys. **6**, 124 (1938).
5. KOMAKI, C., ICHISHIMA, I., KURATANI, K., MIYAZAWA, T., SHIMANOUCHI, T., and MIZUSHIMA, S. M. Bull. Chem. Soc. Japan, **28**, 330 (1955).
6. MIZUSHIMA, S. Structure of molecules and internal rotations. Academic Press, Inc., New York. 1954. p. 42.
7. MIZUSHIMA, S. Structure of molecules and internal rotations. Academic Press, Inc., New York. 1954. p. 98.
8. NATIONAL BUREAU OF STANDARDS (U.S.), Circ. 514, Washington, D.C. (1951).
9. WHITE, J. U., ALPERT, N., and DEBELL, A. G. J. Opt. Soc. Amer. **45**, 237 (1955).

ORGANIC DEUTERIUM COMPOUNDS

XVI. SYNTHESIS OF α-DEUTERATED ALKYL NITRILES[1]

L. C. LEITCH

ABSTRACT

A method of preparing α-deuterated alkyl nitriles by a base-catalyzed exchange with deuterium oxide is described. The products are convenient starting materials for the preparation of α-deuterated acids and alkyl bromides.

Base-catalyzed exchange with deuterium oxide has been used in several instances to deuterate compounds containing labile hydrogen. Thus trichloroethylene-*d* (3), nitromethane-d_3 (4), and nitroethane-1,1-d_2 (2) have been prepared from the protium compounds in this manner. It seemed desirable to determine whether nitriles would equilibrate rapidly enough to make feasible the preparation of α-deuterated nitriles by this method. In nitriles, however, exchange may be accompanied by hydrolysis to the amide or acid. In 1938 Reitz (6) examined the relative rates of exchange and hydrolysis of acetonitrile and found that at 80° C. exchange was about 40 times as rapid as hydrolysis. However the data were obtained using deuterium oxide only slightly enriched in isotopic hydrogen.

It has now been found that acetonitrile exchanges rapidly when heated with a suspension of calcium deuteroxide in heavy water. After five exchanges a product containing 93.4 mol.% CD_3CN and 4.4 mol.% CHD_2CN was obtained.

Propionitrile underwent no significant exchange under the same conditions but at 125° C. appreciable equilibration took place. At higher temperatures, viz. 140° C., considerable amounts of ammonia were formed owing to hydrolysis. *n*-Butyronitrile was exchanged in the same manner to give *n*-butyro-1,1-d_2-nitrile. No significant exchange took place elsewhere in the molecule. Acrylonitrile exchanged rapidly when heated under reflux with the exchange medium but a part of the nitrile polymerized under these conditions. Mass analysis of the product after two exchanges revealed that only one hydrogen atom had been replaced by deuterium, presumably that on the α-carbon atom.

α-Deuterated nitriles are useful starting materials for the synthesis of other deuterated compounds. They are hydrolyzed quantitatively in the presence of hydrogen ions without loss of deuterium by exchange. When nitric acid is used for hydrolysis the organic acid may be recovered in excellent yield as the silver salt. The α-deuterated acids can, of course, also be prepared by decarboxylation of the corresponding deuterated malonic acid (1). However, nitriles are generally more readily available than alkyl malonic acids. The α-deuterated acids undergo brominative decarboxylation to the α-deuterated alkyl bromides (5). These reactions are not particularly useful in the case of acetonitrile because these derivatives are more readily prepared by other methods. However, propionitrile-1,1-d_2 is conveniently converted into ethyl-1,1-d_2 bromide by this means.

[1]*Manuscript received January 9, 1957.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 4248.
Presented at the meeting of the Royal Society of Canada at Toronto in June, 1955.

EXPERIMENTAL

Acetonitrile-d_3

A mixture of acetonitrile (50.0 ml.), deuterium oxide (40 ml.), and calcium deuteroxide (10.0 g.) was heated under reflux for 12 hours under a Stedman column and still head. The exchanged nitrile was distilled over as an azeotrope, b.p. 76° C. The distillate was dried over anhydrous potassium carbonate. Yield: 45.0 ml. It was exchanged twice more with fresh portions of deuterium oxide and calcium deuteroxide, and finally distilled on a vacuum manifold through a U-tube filled with phosphorus pentoxide. The yield of product which showed a small Raman band due to CHD_2CN but none due to CH_3CN or CH_2DCN was 35.0 ml.

A final exchange was carried out with 15 ml. of this product in a sealed tube with a re-entrant joint using 25 ml. of deuterium oxide (99.6%) and 1.0 g. of calcium deuteroxide. After the tube had been heated in a rocking furnace for 12 hours at 85° C., it was opened by means of a magnetic hammer while attached to the vacuum manifold. The nitrile was distilled through a tube containing phosphorus pentoxide and condensed in a trap on the line. The yield of product analyzing 93.4 mol.% CD_3CN was 12.0 ml.

Propionitrile-1,1-d_2

A mixture of propionitrile (25.0 ml.), deuterium oxide (25.0 ml.), and calcium deuteroxide (5.0 g.) was heated in the rocking furnace at 120° C. for 12 hours. When the cold tube was opened no odor of ammonia was apparent; the supernatant layer of exchanged nitrile was distilled off in a vacuum manifold into a trap which contained anhydrous potassium carbonate. Yield: 22.0 ml.

The exchange was repeated until the bands due to CHD and CH_2 stretching vibrations in the Raman had disappeared.

n-Butyronitrile-1,1-d_2

This compound was prepared as described for propionitrile-1,1-d_2.

Acrylonitrile-1-d

A mixture of acrylonitrile (25.0 ml.), deuterium oxide (40.0 ml.), and calcium hydroxide-d_2 (5.0 g.) was heated for 6 hours under reflux with stirring. The nitrile was distilled over as an azeotrope with water, b.p. 70–72° C. The distillate was saturated with anhydrous potassium carbonate and the supernatant layer of nitrile distilled on the vacuum line through a U-tube containing phosphorus pentoxide. Yield: 15.0 ml. (60%). The residue from the azeotropic distillation contained a considerable amount of insoluble polymer. A second exchange gave acrylonitrile-*d* analyzing 96 atom %.

Silver Propionate-1,1-d_2

Propionitrile-1,1-d_2 (8.6 g.) was heated under reflux on the steam bath overnight with 15 ml. of concentrated nitric acid diluted with an equal volume of water. The solution was further diluted to 100 ml. with water and neutralized with 20% aqueous sodium hydroxide. A solution of 35.0 g. of silver nitrate in 125 ml. of water was then added gradually with stirring. After the solution had been left for half an hour the thick white precipitate of silver propionate was filtered off and washed with water. The yield of slightly gray salt after drying in the air in the dark was 22.0 g. (78%).

ACKNOWLEDGMENT

The author is indebted to Dr. W. Cockburn of Defence Research Chemical Laboratories for the analysis of deuterated acrylonitrile and to Dr. H. J. Bernstein of these laboratories for the infrared and Raman spectra.

REFERENCES

1. HOLEMANN, P. and CLUSIUS, K. Ber. **70**, 819 (1937).
2. LEITCH, L. C. Can. J. Chem. **33**, 400 (1955).
3. LEITCH, L. C. and BERNSTEIN, H. J. Can. J. Research, B, **28**, 35 (1950).
4. LEITCH, L. C., GAGNON, P. E., and CAMBRON, A. Can. J. Research, B, **28**, 256 (1950).
5. NOLIN, B. Can. J. Chem. **31**, 1257 (1953).
6. REITZ, O. Z. physik. Chem. A, **83**, 371 (1939).

ORGANIC DEUTERIUM COMPOUNDS

XVII. A SYNTHESIS OF DEUTERATED METHYL CHLOROFORM[1]

J. E. FRANCIS AND L. C. LEITCH

ABSTRACT

1,1,1-Trichloroethane-d_3 has been synthesized from trichloroethylene in four steps via trichloroethylene-*d*, 1,1,1,2-tetrachloroethane-d_2, and 1,1-dichloroethylene-d_2.

The recent interest of spectroscopists in methyl chloroform (1) seemed to justify the preparation of its deuterated analogue. Deuteromethyl chloroform was therefore prepared by the series of reactions outlined below:

$$CHCl{=}CCl_2 \overset{^-OD}{\rightleftharpoons} CDCl{=}CCl_2$$

$$CDCl{=}CCl_2 \overset{DCl}{\rightarrow} CD_2Cl{-}CCl_3$$

$$CD_2Cl{-}CCl_3 \overset{Zn/EtOH}{\rightarrow} CD_2{=}CCl_2$$

$$CD_2{=}CCl_2 \overset{DCl}{\rightarrow} CD_3CCl_3$$

Apart from trichloroethylene-*d*, which was reported earlier by one of us (6), the compounds or their mode of preparation are new. After three equilibrations with alkaline deuterium oxide trichloroethylene-*d* showed no C—H absorption in either the Raman or the infrared. 1,1,1,2-Tetrachloroethane-d_2 was formed when trichloroethylene-*d* was stirred under an atmosphere of deuterium chloride, in the presence of aluminum chloride. Dehalogenation of the tetrachloroethane with zinc dust in ethanol gave 1,1-dichloroethylene-d_2, which had been reported previously (8) but without any mention of the manner in which it was prepared.

Since the addition of hydrogen chloride to 1,1-dichloroethylene had been reported only in patents (3, 7), it appeared desirable to reinvestigate this reaction before exposing easily polymerized deuterated dichloroethylene to the combined action of deuterium chloride and anhydrous aluminum chloride. Contrary to reports in the patent literature (2, 4), 1,1-dichloroethylene could not be prepared by dehydrochlorination of 1,1,1-trichloroethane. However, the isomeric 1,1,2-trichloroethane reacted smoothly with aqueous calcium hydroxide to give a satisfactory yield of 1,1-dichloroethylene.

Hydrogen chloride added without too much difficulty to 1,1-dichloroethylene in the presence of anhydrous aluminum chloride. The addition of deuterium chloride to 1,1-dichloroethylene-d_2 carried out in the same manner gave a moderate yield of 1,1,1-trichloroethane-d_3 or deuterated methyl chloroform.

In the belief that it would also be of interest to obtain the semideuterated compounds $CHDClCCl_3$ and $CHD{=}CCl_2$, the addition of deuterium chloride to trichloroethylene was carried out under the same conditions. The Raman spectrum of the product indicated it was a mixture of all three tetrachloroethanes—CH_2ClCCl_3, $CHDClCCl_3$, and CD_2Cl-CCl_3. Apparently, the presence of anhydrous aluminum chloride had catalyzed hydrogen–

[1]*Manuscript received January 9, 1957.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 4252.
Presented at the meeting of the Royal Society of Canada at Montreal in June, 1956.

deuterium exchange as well as addition to the double bond. In the aromatic series this reaction is known, having been employed by Klit and Langseth (5) to prepare benzene-d_6 from benzene and deuterium chloride. However its occurrence with olefins is not believed to have been noted before.

EXPERIMENTAL

Trichloroethylene-d

A mixture of trichloroethylene (25 ml.), deuterium oxide (25 ml.), and calcium deuteroxide (4.0 g.) (or an equivalent amount of freshly calcined calcium oxide) was heated with stirring under gentle reflux for 12 hours in a 100 ml. round-bottomed flask surmounted by a Vigreux column and still head. In some experiments the desiccant in the tube attached to the still head was blown out and a deposit of carbon was formed in the column. This was probably produced by the spontaneous combustion of traces of chloroacetylene formed by dehydrochlorination of the trichloroethylene. The exchanged trichloroethylene was then distilled off as an azeotrope, collected in a separatory funnel. After it was dried over anhydrous potassium carbonate the exchange was repeated with fresh portions of deuterium oxide and calcium deuteroxide (or calcium oxide). After three exchanges the trichloroethylene-*d*, n_D^{20} 1.4771, contained only traces of the normal compound, n_D^{20} 1.4776 according to its nuclear magnetic resonance spectrum. The yield was nearly quantitative.

The deuterium oxide recovered from each exchange was re-used to enrich a second run of trichloroethylene.

Deuterium Chloride

The gas was generated by adding deuterium oxide dropwise to phosphorus pentachloride in an evacuated round-bottomed flask. It was freed of phosphorus halides by being cooled to −78° C. in a spiral trap immersed in dry ice and acetone and collected in a 2 l. evacuated gas bulb. The entire apparatus was flushed out once with deuterium chloride to remove hydrogen adsorbed as water on the walls.

1,1,1,2-Tetrachloroethane-d_2

Trichloroethylene-*d* (25 ml.) and anhydrous aluminum chloride (2.0 g.) were placed in a 100 ml. round-bottomed flask, a small magnetized bar sealed in glass was introduced for stirring, and the flask was attached to the deuterium chloride generator by an adapter. After cooling and evacuating, stirring was started and deuterium chloride introduced from the gas bulb until the pressure was nearly one atmosphere. Addition of deuterium chloride took place slowly over 2 days, a total of about 4 l. being consumed.

The reaction mixture was connected to a stock trap cooled to −78° on a vacuum line and distilled. The distillate (24.5 ml.) was fractionated in a column packed with glass helices. The pure product, b.p. 128–129° C., n_D^{20} 1.4815, amounted to 23.0 g. (58% of the theoretical amount). Its Raman spectrum showed only very faint absorption in the C—H region.

1,1-Dichloroethylene

Dehydrochlorination of 1,1,1-trichloroethane by means of calcium hydroxide was unsuccessful. A suspension of 20 g. of the trichloroethane in 50 ml. of water to which 5.0 g. of calcium oxide had been added was heated under reflux for 5 hours without obtaining a trace of 1,1-dichloroethylene. With 1,1,2-trichloroethane, however, yields of

75–80% 1,1-dichloroethylene were readily obtained. The product was dried by distillation through a U-tube containing phosphorus pentoxide on the vacuum line. To prevent polymerization the monomer was treated with a few crystals of hydroquinone.

1,1,1-Trichloroethane

1,1-Dichloroethylene (5.0 ml.) and 0.05 g. of anhydrous aluminum chloride were stirred in a 100 ml. round-bottomed flask under an atmosphere of hydrogen chloride at 20° C. After 1½ hours no more hydrogen chloride was absorbed. The product was distilled out into a trap on the vacuum line. Yield: 7.5 g. Fractional distillation in a column packed with glass beads gave 5.0 g. of 1,1,1-trichloroethane, b.p. 72–74° C., n_D^{20} 1.4384.

1,1-Dichloroethylene-d_2

To a boiling stirred suspension of 28 g. of zinc dust in 50 ml. of 95% ethanol in a 250 ml. round-bottomed flask with a reflux condenser to which was attached a spiral trap cooled in dry ice and acetone to −78° C. was added dropwise over a period of 2 hours 24.8 g. of 1,1,1,2-tetrachloroethane-d_2. After addition of the halide the dichloroethylene was swept out of the apparatus by a slow current of nitrogen. The product in the spiral trap was distilled through a tube containing phosphorus pentoxide on the vacuum line. The yield of pure product was 12.8 g. (89%).

1,1,1-Trichloroethane-d_3

In a 500 ml. round-bottomed flask were placed a magnetized bar and 107 mg. of pulverized anhydrous aluminum chloride. The flask was pumped out thoroughly and then 6.2 ml. at 0° C. of 1,1-dichloroethylene-d_2 was distilled into the flask from a trap. One atmosphere of deuterium chloride was then introduced and stirring started. After a short induction period absorption began. The flask was immersed in water at 18° C. to prevent polymerization. After about 1 hour absorption of deuterium chloride ceased; the reaction mixture was purple. The product was distilled *in vacuo* as described for the light compound. Yield: 8.5 g. (78%). It was purified by fractional distillation in a column packed with glass helices, b.p. 73–74° C., n_D^{20} 1.4370. Yield: 4.7 g.

1,1,1,2-Tetrachloroethane-d

Deuterium chloride was reacted with trichloroethylene as described for the synthesis of the dideuterated compound. The Raman spectrum of the product showed absorption due to CH_2 and CD_2 groups as well as the CHD group.

REFERENCES

1. Evans, J. C. and Bernstein, H. J. Can. J. Chem. **33**, 1746 (1955).
2. Howell, W. N. Imp. Chem. Ind. Brit. Patent No. 534,733 (March 17, 1941); Chem. Abstr. **36**, 1336 (1942).
3. I. G. Farbenind. Ger. Patent No. 523,436 (April 23, 1931); Chem. Abstr. **25**, 3362 (1931).
4. I. G. Farbenind. Brit. Patent No. 349,872 (July 2, 1931); Chem. Abstr. **26**, 5314 (1932).
5. Klit, A. and Langseth, A. Z. physik. Chem. A, **176**, 65 (1936).
6. Leitch, L. C. and Bernstein, H. J. Can. J. Research, B, **28**, 35 (1950).
7. Nutting, H. S. and Huscher, M. E. Dow Chem. Co., U.S. Patent No. 2,209,000 (July 23, 1940); Chem. Abstr. **35**, 140 (1941).
8. Verhulst, J., Jungers, J. C., and de Hemptinne, M. Natuurw. Tijdschr. (Belg.), **22**, 56 (1940); Chem. Zentr. I, 1809 (1941).

SOME ACENAPHTHENE COMPOUNDS[1]

E. H. CHARLESWORTH, H. CAMPBELL, J. J. CONN, C. T. ELSTON, AND D. L. STACHIW

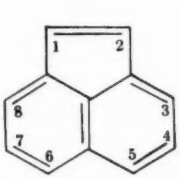
ABSTRACT

A new geometric isomer of 1,2-dichloroacenaphthene has been isolated among the reaction products of acenaphthylene and hypochlorous acid. Some new derivatives of acenaphthenone and acenaphthenol are reported.

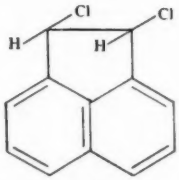
INTRODUCTION

A number of students have worked at times in these laboratories in attempts to make the chlorohydrin and epoxide of acenaphthylene (I). This work has not met with success, but a number of compounds containing the acenaphthene nucleus have been prepared, which have been thought worthy of record. In attempts to make the chlorohydrin by treatment of acenaphthylene with hypochlorous acid by the method of Detoeuf (2), a new dichloroacenaphthene, m.p. 64° C., has been isolated. This is undoubtedly a geometric isomer of the 1,2-dichloroacenaphthene of melting point 115° C. prepared by Campbell (1) by the direct chlorination of acenaphthylene in carbon tetrachloride.

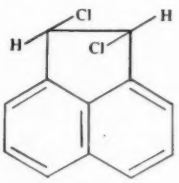
The presence of the chlorine in the 1,2-positions of the 64° melting compound has been established by oxidation with dichromate to naphthalic anhydride. Attempts to establish the configuration of these two isomeric 1,2-dichloroacenaphthenes (II and III) have been unsuccessful. Both the isomers on hydrolysis gave a mixture of the two corresponding glycols. Although the configuration of the glycols has been established by Jack and Rule (8), we have not been able to prepare either of the chlorides from the glycols with the usual agents such as zinc chloride – hydrochloric acid, phosphorus trichloride, or thionyl chloride. Both the dichloroacenaphthenes on treatment with potassium acetate and acetic acid were converted to the monoacetate of the *cis*-glycol.



I



II



III

A study of the methylene group in acenaphthenone (IV) has been undertaken by a series of condensations with aromatic aldehydes under alkaline (Claisen-Schmidt) and acid conditions. This reaction has been studied previously by two groups of workers, de Fazi (3) in Italy and Sircar and Gopalan (10, 11, 12) in India. In several cases, as shown in Table I, there is disagreement between these authors on the melting point of the isolated product. This suggested that geometric isomers (V and VI) may have been isolated.

In attempts to clarify the situation, we have always isolated, with one exception, one of the described forms only, no matter whose directions we followed. When *o*-nitrobenzaldehyde was employed, both the described condensation products were isolated,

[1]*Manuscript received December 3, 1956.*
Contribution from the Department of Chemistry, University of Manitoba, Winnipeg, Manitoba.

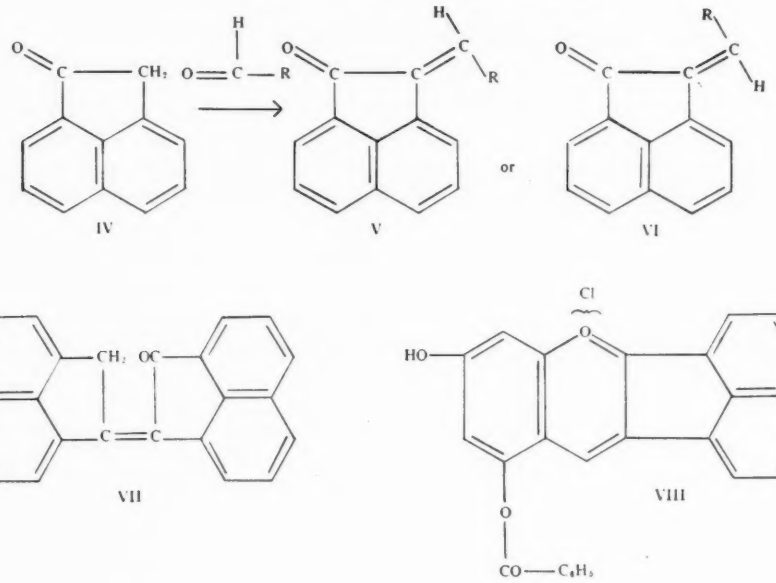


TABLE I

$C_{10}H_{10}$(CO)C=CH.R COMPOUNDS FROM ACENAPHTHENONE AND R.CHO

R—C(H)=	Melting point	
	Literature	This investigation
p-Nitrobenzal	202–203° (3) 239–240° (12)	— 241°
o-Nitrobenzal	241–243° decomp. (3) 157° (11)	242–244° 163–165°
p-Methoxybenzal (anisal)	95–98° (1) 126–127° (12)	95–96° —
o-Hydroxybenzal (salicylal)	168° (1) 186–187° (10)	— 185–186°
Cinnamal	167–168° (12) 214–215° (1)	163–165° —
o-Methoxybenzal	Not listed	192–192.5°
5-Nitrosalicylal	Not listed	242–243° decomp.
p-Hydroxybenzal	Not listed	215–216°

the higher melting under alkaline conditions and the lower melting under acid conditions. That the two products were geometric isomers and not polymorphic forms was shown by the difference in the ultraviolet absorption curves. The characteristic peak at 281–282 mμ of the 164° melting compound was missing in the 245° melting compound.

The expected condensation products have been obtained from the three new aldehydes listed in Table I. Naphthalic acid or its anhydride, or biacenone (VII), was the only isolated product, when condensations were carried out with *p*-methylbenzaldehyde, *m*-methylbenzaldehyde, and *m*-methoxybenzaldehyde. Biacenone also resulted when aliphatic aldehydes were employed. This is quite in agreement with the work of Graebe and Gfeller (6), who reported the formation of naphthalic acid by boiling acenaphthenone with sodium hydroxide solution, and of Graebe and Jequier (7), who reported the formation of biacenone from self-condensation of acenaphthenone in alcoholic sodium hydroxide even at room temperature. Phloroglucinaldehyde does not react with acenaphthenone under the normal conditions, but condenses with itself. However, using 2-O-benzoylphloroglucinaldehyde, the pyrylium salt (VIII) was produced.

The semicarbazone of acenaphthenone, which does not seem to have been previously reported, was prepared in the usual manner. A number of characterization derivatives of acenaphthenol, the 3,5-dinitrobenzoate, the *p*-nitrobenzoate, and the α-naphthylurethan, have been prepared.

EXPERIMENTAL

Acenaphthenol acetate, acenaphthenol, and acenaphthenone were prepared as described by Fieser and Cason (4). Acenaphthene (Reilly Tar and Chemical Corporation) was oxidized with lead tetracetate. Alkaline hydrolysis of the resulting acetate gave acenaphthenol, which on oxidation with chromic oxide and acetic acid yielded crude acenaphthenone. It was purified by steam distillation and finally by crystallization from ethanol, as colorless needles which melted at 121° C.*

Acenaphthylene

This substance was prepared essentially as described by Flowers and Miller (5) by passing acenaphthenol acetate vapors and nitrogen through a quartz tube heated to 520° by a nichrome heating element. The crude reddish solid pyrolysis product was washed well with water to remove acetic acid and crystallized several times from ethanol (charcoal). It was thus obtained as lemon yellow plates, m.p. 92° C. in 80% yield. The purification of the crude product could also be somewhat simplified by distillation *in vacuo*, using hydroquinone as a polymerization inhibitor and collecting the fraction boiling at 135–140° (15 mm.). The yields are somewhat lower owing to partial polymerization of the acenaphthylene.

The optimum rate of flow of the acetate through the pyrolysis tube appeared to be when the issuing vapors were thin and pale orange in color. If the vapors were heavy and bright yellow in color, it indicated that unpyrolyzed acetate was passing through the tube. On the average about 2 hours were required to put each 100 g. of the acetate through the tube. The efficiency of the tube was reduced somewhat by carbonaceous material and for best results it should be cleaned after every run of 200 g. To prevent oxidation and photochemical polymerization, the acenaphthylene was stored in amber glass stoppered bottles and kept in the dark.

1,2-Dichloroacenaphthene (m.p. 64°) (II or III)

A variety of unsuccessful attempts were made to obtain acenaphthylene chlorohydrin by the action of hypochlorous acid on acenaphthylene under a variety of conditions.

**Melting points have been determined by the capillary tube method and are uncorrected.*

In several cases the above compound of melting point 64° was isolated. A description follows of the method that seemed to give the largest amount of this substance.

Acenaphthylene (25 g.), monochlorourea solution (250 ml., 9.9% in hypochlorous acid, prepared by the method of Detoeuf (2)), and glacial acetic acid (15 ml.) were placed in a flask (500 ml.) and stirred mechanically. The reaction proceeded very slowly; after 24 hours a gummy solid had collected on the walls of the flask and the loss in hypochlorous acid was about 10% greater than the spontaneous decomposition of the acid. After 5 days the hypochlorous acid concentration was nil. The contents of the flask were then extracted with ether and washed with water and a 5% solution of sodium bicarbonate. The brown oil left after drying and removal of the ether was distilled *in vacuo*. A light yellow oil (14.7 g.) was collected at 170–180° (15 mm.). After the oil had been left for several days in the ice chest, a crop of light yellow crystals (4.8 g.) was obtained. On recrystallization from ethanol these were obtained as fine white needles which melted sharply at 64° C. Found: Cl, 31.83%. Calculated for $C_{12}H_8Cl_2$: Cl, 31.79%. It would seem that this substance is a geometric isomer of the 1,2-dichloroacenaphthene, melting point 115° C., prepared by Campbell (1) by the chlorination of acenaphthylene in carbon tetrachloride solution.

The residue oil, from which the 64° melting crystals were obtained, was distilled *in vacuo*. The yellow oil, b.p. 165–170° (14 mm.), did not crystallize. Found: Cl, 19.93%. Calculated for $C_{12}H_7Cl$: Cl, 19.02%. It would seem that this oil was probably identical with 1-monochloroacenaphthylene, which Campbell (1) prepared by treatment of the 115° melting dichloride with an alcoholic solution of sodium ethylate. With a saturated solution of picric acid in ethanol, the oil gave an orange picrate, m.p. 152–153°. A mixed melting point of this picrate and a sample of the picrate, m.p. 151–152°, prepared from a sample of Campbell's oil gave no depression.

Oxidation of the Dichloroacenaphthene

The 64° melting dichloroacenaphthene (2.0 g.) and sodium dichromate (10 g.) were dissolved in glacial acetic acid, 30 ml., and refluxed for 4 hours. The mixture was diluted with water (150 ml.) and the resulting precipitate filtered off. It was triturated with sodium bicarbonate solution and filtered. The filtrate was decolorized with charcoal and acidified with hydrochloric acid. The resulting precipitate was further purified by a repetition of the process and finally crystallized from ethanol. There was thus obtained a white, halogen-free, crystalline solid which melted at 268–270° and which gave a blue fluorescence in concentrated sulphuric acid. This substance proved to be naphthalic acid and its isolation can be accepted as proof that the halogens in the dichloro compound are in the 1,2-positions. The 115° melting isomer also produced naphthalic acid on oxidation.

Acenaphthylene Glycols

Authentic samples of the two glycols, the *cis*, m.p. 206–208°, and the *trans*, m.p. 155–156°, were prepared by the hydrolysis of the 115° melting 1,2-dichloroacenaphthene according to the directions of Jack and Rule (8). Some of the *cis*-glycol was also prepared by the oxidation of acenaphthene with selenium dioxide following the method of Monti (9).

Hydrolysis of the 64° melting dichloroacenaphthene gave both the *cis*- and *trans*-glycols.

The 64° melting dichloro compound (1.0 g.) was added to a solution of potassium acetate (2 g.) in glacial acetic acid (4 ml.). After the mixture was refluxed for 1 hour it was diluted with water. The resulting solid was crystallized three times from ethanol (charcoal) and gave fine white needles, m.p. 123° C. These were identical with the monoacetate of the *cis*-glycol. The 115° melting dichloro compound on being subjected to similar treatment yielded the same monoacetate of the *cis*-glycol.

Attempted Reactions of Glycols with Halogenating Agents

Various attempts to form the corresponding halogen compounds from the known glycols with agents such as concentrated hydrochloric acid and zinc chloride, phosphorus trichloride, and thionyl chloride (with and without pyridine) failed to yield the halogen compounds or any other identifiable material.

Derivatives of Acenaphthenol and Acenaphthenone

3,5-Dinitrobenzoate

This was prepared by treatment of acenaphthenol in pyridine with 3,5-dinitrobenzoyl chloride in the usual manner. On crystallization from acetone (charcoal) the product was obtained as small pale yellow needles which melted at 166° C. Found: C, 62.4; H, 3.30%. Calculated for $C_{19}H_{12}O_6N_2$: C, 62.6; H, 3.32%.

p-Nitrobenzoate

This ester was prepared in the same manner and crystallized from ethanol (charcoal) in fine pale yellow needles, m.p. 117–118° C. Found: C, 71.5; H, 4.13%. Calculated for $C_{19}H_{13}O_4N$: C, 71.4; H, 4.10%.

α-Naphthylurethan

α-Naphthyl isocyanate was allowed to react with acenaphthenol dissolved in dry pyridine. On crystallization from pyridine, the urethan was obtained as long colorless prisms which melted at 206° with decomposition. Found: C, 81.2; H, 5.06%. Calculated for $C_{23}H_{17}O_2N$: C, 81.4; H, 5.05%.

Acenaphthenone Semicarbazone

Acenaphthenone was allowed to react in the usual manner in hot alcoholic solution with semicarbazide hydrochloride and sodium acetate. The crude yellow semicarbazone which separated was recrystallized twice from ethanol (charcoal) and was obtained as a white fluffy solid, m.p. 241–243°, with decomposition. Found: N, 18.6%. Calculated for $C_{13}H_{11}ON_3$: N, 18.7%.

o-Methoxybenzalacenaphthenone

Acenaphthenone (1 g.) and *o*-methoxybenzaldehyde (0.81 g.) were dissolved in ethanol (50 ml.) and a solution of sodium hydroxide (5 g. in 5 ml. of water) was added slowly over a period of 2 hours. A yellow precipitate started to form during the addition of the alkali. After 24 hours at room temperature, the precipitate was filtered off, washed with ethanol, and crystallized from a mixture of chloroform and ethanol. It separated in yellow prisms (1.2 g.) which melted at 192–193° C. Found: C, 83.8; H, 4.96%. Calculated for $C_{20}H_{14}O_2$: C, 83.9; H, 4.93%.

5-Nitrosalicylalacenaphthenone

This was prepared according to the above directions. The crude dark red solid was crystallized from acetic acid (charcoal) and gave fluffy orange crystals, m.p. 242–243°

with decomposition. Found: C, 71.6. H, 3.53%. Calculated for $C_{19}H_{11}O_4N$: C, 71.9; H, 3.50%.

p-Nitrobenzalacenaphthenone

(1) When this was prepared as described above, which is the method of de Fazi (3), it was obtained as fine yellow needles, m.p. 242° C. This was the melting point of the product made by Sircar and Gopalan. The product of melting point 202–203° described by de Fazi was not obtained.

(2) If prepared according to the directions of Sircar and Gopalan (10, 11, 12) using 10% alcoholic potassium hydroxide as condensing agent, the product again melted at 241° C. Even with variation in temperature of condensation from −20 to +80° C. this product was always obtained under alkaline conditions.

(3) Acenaphthenone (1 g.) and *p*-nitrobenzaldehyde (0.95 g.) were dissolved in glacial acetic acid (10 ml.) and dry hydrogen chloride gas was passed through the mixture at room temperature over a period of 1 hour. The yellow condensation product started to precipitate immediately on the addition of the gas. At the end of the reaction it was filtered off, washed with dilute acetic acid, and crystallized from glacial acetic acid. It again melted at 241° C.

o-Nitrobenzalacenaphthenones

When produced by either of the alkaline condensation conditions described above, it was obtained as very fine yellow needles, m.p. 242–244°. When they were cooled and remelted there was no change in the melting point.

When formed by condensation with hydrogen chloride, yellow needles, m.p. 163–165°, resulted, which, after being cooled, remelted without change. That the two substances were geometric isomers and not polymorphic forms was indicated by the ultraviolet absorption curves. The peak at 281–282 mμ exhibited by the lower melting compound was not present in the higher melting. Both had the same sort of peak at 231–232 mμ.

Attempts to convert the lower melting isomer to the higher by use of ultraviolet light, iodine, heat, or palladized charcoal resulted in recovery of the starting material in the first two cases, and a dark uncrystallizable oil in the latter two. The low melting form if allowed to stand with alcoholic potash yielded, after acidification, naphthalic anhydride.

p-Hydroxybenzalacenaphthenone

This product resulted from both alkaline and acid condensations. It was recrystallized from acetic acid and melted at 215–216° C. Found: C, 83.7; H, 4.16%. Calculated for $C_{19}H_{12}O_2$: C, 83.8; H, 4.43%.

Salicylalacenaphthenone

Alkaline condensations gave the product melting at 185–186° C.

p-Anisalacenaphthenone

The product of melting point 95° was isolated by either of the alkaline methods of condensation. Nothing was isolated under the acid conditions.

Attempted Condensations

Normal condensations failed to occur with acenaphthenone under alkaline conditions and the following substituted benzaldehydes, *m*-methoxy-, *m*-methyl-, and *p*-methyl-.

In the first two cases either naphthalic acid or its anhydride was isolated and in the latter case self-condensation of the acenaphthenone gave yellow crystals of biacenone, m.p. 260° C. Found: C, 90.3; H, 4.43%. Calculated for $C_{24}H_{14}O$: C, 90.5; H, 4.43%. Biacenone was the only isolated product when acenaphthenone was treated with aliphatic aldehydes (propionic and butyric).

Acenaphthyleno-1',2';2,3,5-benzoyloxy-7-hydroxybenzopyrilium Chloride

Acenaphthenone (1 g.) and 2-O-benzoylphloroglucinaldehyde (1.5 g.) were dissolved in anhydrous ethyl acetate (5 ml.) and a few drops of glacial acetic acid (to effect complete solution of the aldehyde). Dry hydrogen chloride gas was passed into the mixture for an hour. The solution, which assumed a deep red coloration, was placed in an ice box for 4 or 5 days. A red powdery material separated which melted at 140–141°. To recrystallize this material, it was dissolved in a little absolute ethanol and the solution was again saturated with hydrogen chloride gas. Found: C, 68.3; H, 4.00; Cl, 7.29; loss at 110° *in vacuo* over phosphoric anhydride 6.45%. Calculated for $C_{26}H_{15}O_4Cl.1.5H_2O$: C, 68.9; H, 3.98; Cl, 7.84; H_2O, 5.97%.

ACKNOWLEDGMENT

The authors are grateful to the National Research Council of Canada for grants and bursaries which have aided in this research.

REFERENCES

1. CAMPBELL, B. A. J. Chem. Soc. **107**, 918 (1915).
2. DETOEUF, A. Bull. soc. chim. (4), **31**, 102 (1922).
3. FAZI, R. DE. Gazz. chim. ital. **54**, 658 (1924).
4. FIESER, L. F. and CASON, J. J. Am. Chem. Soc. **62**, 432 (1940).
5. FLOWERS, R. G. and MILLER, H. F. J. Am. Chem. Soc. **69**, 1388 (1947).
6. GRAEBE, C. and GFELLER, E. Ann. **276**, 1 (1893).
7. GRAEBE, C. and JEQUIER, J. Ann. **290**, 195 (1896).
8. JACK, K. M. and RULE, H. G. J. Chem. Soc. 188 (1938).
9. MONTI, L. Gazz. chim. ital. **68**, 608 (1938).
10. SIRCAR, A. C. and GOPALAN, M. D. R. J. Indian Chem. Soc. **9**, 103 (1932).
11. SIRCAR, A. C. and GOPALAN, M. D. R. J. Indian Chem. Soc. **9**, 297 (1932).
12. SIRCAR, A. C. and GOPALAN, M. D. R. J. Indian Chem. Soc. **9**, 639 (1932).

AN OXYGENATED FATTY ACID FROM THE SEED OIL OF HIBISCUS ESCULENTUS[1]

MARY J. CHISHOLM AND C. Y. HOPKINS

ABSTRACT

The fatty acids of okra seed oil (*Hibiscus esculentus* L.) were examined. Acetylation of the oil, followed by saponification and separation of the acids, gave 12,13-dihydroxyoleic acid. From this and other evidence it is concluded that 12,13-epoxyoleic acid is present as a constituent of the glycerides. An oxygenated acid has not been identified previously in seed oils of this plant family (Malvaceae). 9-Hexadecenoic acid was also found. The total fatty acid composition was determined by distillation of the methyl esters and analysis of the distilled fractions. The percentages of the acids identified were estimated as follows: 9-hexadecenoic 0.6, palmitic 29, linoleic 39, oleic 23, stearic 2, 12,13-epoxyoleic 3, arachidic <1. Myristic, eicosenoic, and trienoic acids were not detected and, if present, are judged to have been in amounts less than 1% of the total.

INTRODUCTION

Various plants of the family Malvaceae produce oleaginous seeds but only the cottonseed (*Gossypium* sp.) is utilized commercially for its oil. However, okra (*Hibiscus esculentus* L.) has been proposed as an oilseed crop since it can be grown in areas that are unsuitable for cotton.

The fatty acids of okra seed oil have been examined by several groups of workers (3, 4, 6, 7, 10) and it is agreed that the major components are palmitic, oleic, and linoleic acids. In addition, it was observed by Clopton, Roberts, and Jeskey (3) that their sample of okra oil contained an unusual acid, apparently of greater chain length than C_{18}. They estimated that this acid constituted 8% of the total fatty acids and suggested that it was probably an unsaturated 20-carbon acid. Accordingly, the present work was undertaken to determine whether this might have been eicosenoic acid, particularly as there has been no other indication that eicosenoic acid occurs in any species of Malvaceae.

EXPERIMENTAL

Properties of the Oil

Okra seed was obtained from commercial seed houses. Two varieties were examined, viz. White Velvet and Perkins Mammoth Long Pod. The seeds were ground in a Wiley mill, the moisture content of the meal was determined, and the oil was extracted with petroleum ether. The yields of oil and the analytical values are shown in Table I.

The oil of the Perkins variety was examined in detail. A portion was saponified and the total fatty acids were freed from unsaponifiable matter. Their ultraviolet absorption, before and after alkali isomerization, is shown in Fig. 1.

The content of conjugated acids before isomerization was quite small and the total content of triene acid was negligible.

Examination of the Methyl Esters

The oil (Perkins variety) was converted to methyl esters by methanolysis with dry hydrogen chloride as catalyst. The glycerol was removed by washing with water and

[1]*Manuscript received December 19, 1956.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada. Presented at the Annual Conference of the Chemical Institute of Canada on May 30, 1956.
Issued as N.R.C. No. 4253.

TABLE I

OKRA SEED OIL

	White Velvet	Perkins Mammoth Long Pod
Oil content of seed, % (at 10% moisture)	12.4	16.9
Iodine value	85.4	93.5
Saponification value	194.5	192.7
Unsaponifiable, %	0.7	0.7
Acetyl value		7.8
Oxirane oxygen, %		0.2*
Peroxide value		5.6
Acid value	0.7	1.0
Halphen test	Positive	Positive
Refractive index, 25°		1.4699

*Method of Swern (11).

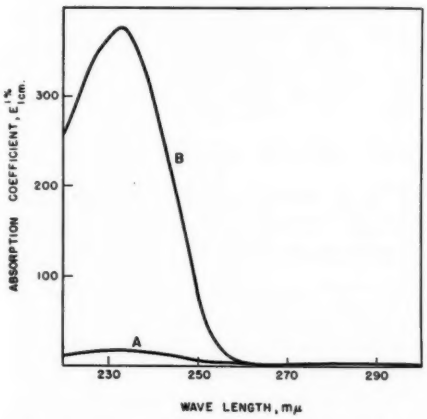


FIG. 1. Ultraviolet spectrum of okra oil fatty acids freed of unsaponifiable matter. A, before isomerization; B, after isomerization (in glycerol 45 minutes at 180°).

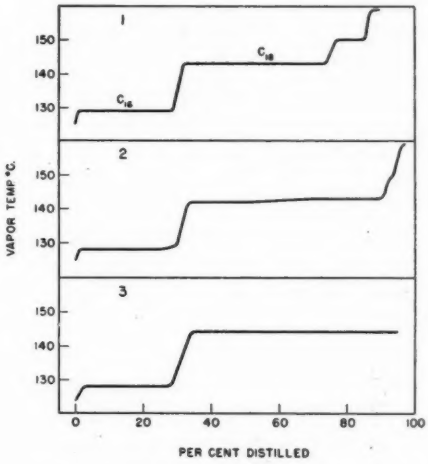


FIG. 2. Distillation of methyl esters: 1, esters made from the whole oil; 2, esters freed of oxygenated acid; 3, esters from oil freed of oxygenated acid and hydrogenated.

the esters were dried. The mixed esters (318 g.) were distilled through a Podbielniak Heli-Grid column at a pressure of 0.5 mm., with the addition of a heavy petroleum fraction as a chaser. The usual C_{16} and C_{18} fractions were collected but the subsequent fractions were abnormal. A considerable portion distilled at a temperature between that of the ordinary C_{18} and C_{20} esters (Fig. 2, distillation 1). (This was doubtless the fraction that led the earlier workers (3) to suspect the presence of a C_{20} acid.) This fraction had an appreciable acetyl value and accordingly was thought to contain an oxygenated acid.

A fresh lot of okra oil was therefore treated by the procedure of Gunstone (5) to remove any epoxy or hydroxy acids by acetylation, hydrolysis, and solvent partition. The remaining mixed acids were esterified with methanol and distilled. Only the usual C_{16} and C_{18} esters distilled and there was no abnormal fraction (Fig. 2, distillation 2).

A further quantity of the oil was freed of oxygenated acids, hydrogenated, converted to methyl esters, and distilled through a spinning band column (distillation 3). The percentage composition of the non-oxygenated acids, by chain length, was estimated from this distillation as follows: C_{16}, 30%; C_{18}, 65%; undetermined, 5%.

TABLE II

DISTILLATION OF METHYL ESTERS
(Freed of oxygenated acid)

Fraction	Temp., °C. (0.5 mm.)	Weight, g.	Chain length	Iodine value	Refractive index, 25°
1	128	6.6	C_{16}	13.9	
2	128	2.8		7.7	
3	128	32.3		0.9	
4	128	12.4		0.1	
5	128–129	12.0		4.5	
6	129–141	6.4		105.3	1.4527
7	141–142	2.2	C_{18}	145.9	1.4577
8	142	43.1		153.8	1.4582
9	142–143	37.0		145.8	1.4573
10	143	24.6		128.4	1.4557
11	143	10.1		90.9	1.4518
12	143	8.0		78.6	
13	143–147	5.2		55.8	
14	147–149	4.0		41.4	
15	149–158	4.0		59.5	
16	158–159	2.4	C_{20}	94.8	

The data from distillation 2 are given in Table II. A number of the fractions were examined individually to identify the component acids. These are shown in Table III. Identity of the acid or its derivative was confirmed by mixed melting point in each case.

TABLE III

IDENTIFICATION OF ACIDS

Fraction	Acid	Identified as:	M.p., °C.
1	9-Hexadecenoic	9,10-Dihydroxypalmitic	124–125
3	Palmitic	Palmitic	62–62.5
8	Linoleic	Diepoxystearic	78–79
11	Oleic	Dihydroxystearic	129–130
13	Stearic	Stearic	69–70
			Equiv. wt. 285
—	12,13-Epoxyoleic	See text	
15	Arachidic	Methyl arachidate	45.5–46.5

Fractions 1 and 2 were combined and recrystallized from acetone at −40°. The crystals were saponified giving palmitic acid. The ester from the filtrate was saponified and hydroxylated by alkaline permanganate, giving 9,10-dihydroxypalmitic acid. Thus the low-boiling material consists mainly of esters of hexadecenoic and palmitic acids. Myristic acid was not identified although there was a small forerun. Fractions 3, 4, and 5 were almost pure methyl palmitate.

Fraction 8 was mainly methyl linoleate. On being saponified and treated with peracetic acid, it gave diepoxystearic acid, m.p. 78–79°. Oleic acid was identified in fraction 11 and stearic acid in fraction 13.

Fraction 15 was recrystallized from acetone at $-10°$ and again from ethanol, giving methyl arachidate. The acetone filtrate was examined but did not yield any eicosenoate, nor did fraction 16. It is estimated that any eicosenoic acid in excess of 1% of the total fatty acids would have been found in this procedure. Examination of the distillation residue indicated the presence of minor amounts of higher saturated acids ($>C_{20}$).

Isolation of an Oxygenated Acid

Freshly-extracted okra seed oil (125 g.) was refluxed with five volumes of glacial acetic acid for 6 hours (5). The acetic acid was distilled off under reduced pressure and the residual oil was saponified by alcoholic potassium hydroxide. Most of the alcohol was removed and replaced by water. The solution was extracted with petroleum ether to remove unsaponifiable matter and then acidified to liberate the fatty acids.

The mixed acids were dissolved in petroleum ether and shaken with successive portions of aqueous methanol (four volumes CH_3OH: one volume H_2O). From the methanol extracts there was obtained 5.3 g. of crude oxygenated fatty acid. It was recrystallized from 60 ml. of acetone at $-30°$ (yield 3.0 g.) and then from petroleum ether:ethyl ether (3:1) at room temperature. The product (2.5 g.) melted at 53.5–54.5° and gave the following analysis: equivalent weight 316.4; OH, 10.85, 10.65; C, 68.99; H, 11.05 (calc. for dihydroxyoctadecenoic acid, $C_{18}H_{34}O_4$: equivalent weight 314.4; OH, 10.82; C, 68.75; H, 10.89). The iodine value was 103.0, somewhat higher than calculated for one double bond; there is evidently some absorption of iodine by reaction with the OH groups.

The analysis shows, therefore, that the substance is almost certainly a dihydroxyoctadecenoic acid.

Determination of Structure

The acid was split by oxidation with potassium permanganate in acetic acid. A solution of 2.9 g. of the dihydroxyoctadecenoic acid in 30 ml. of glacial acetic acid was held at 40–50° while 8 g. of powdered $KMnO_4$ in portions was added with stirring. The mixture was kept at 50° for a further 3 hours. The resulting products, isolated by the usual procedures, were azelaic acid, m.p. 104–106°, and hexanoic acid, the latter identified as the *p*-phenylphenacyl ester, m.p. 64–65°. These melting points were not changed by admixture with known samples.

Another portion of the dihydroxyoctadecenoic acid was hydrogenated in methanol with Adams' catalyst, giving dihydroxystearic acid, m.p. 96.5–97°, from ethyl acetate. Oxidative fission by permanganate–periodate (8) gave dodecanedioic acid, m.p. 127°; equivalent weight, 115.8 (theory 115.2).

The original dihydroxy acid is therefore 12,13-dihydroxy-9-octadecenoic acid (12,13-dihydroxyoleic acid). Its identity was subsequently confirmed by mixed melting point with a sample kindly supplied by Dr. F. D. Gunstone. The identity of the 12,13-dihydroxy stearic acid was confirmed in the same way.

A portion of the original dihydroxyoleic acid was hydroxylated by alkaline permanganate and gave tetrahydroxystearic acid, m.p. 164°. This proved, by mixed melting point, to be identical with the erythro-9,10-threo-12,13-tetrahydroxystearic acid of Bharucha and Gunstone (1).

Identification of the Epoxy Group by Nuclear Magnetic Resonance Spectra

9,10-Epoxystearic acid and 12,13-epoxyoleic acid were prepared by the action of peracetic acid on oleic and linoleic acids, respectively. The 12,13-epoxyoleic acid was

freed from diepoxy acid by fractional crystallization from acetone at low temperature. It had neutralization equivalent 297.3 (theory 296.4).

The proton magnetic resonance spectra of okra seed oil, oleic acid, and the two monoepoxy acids were obtained with a Varian NMR spectrometer at 40 megacycles; the samples were in solution in chloroform, with the exception of the oleic acid which was examined in the pure liquid form. The results are shown in Figs. 3 and 4.

The signal in the vicinity of 60 cycles per second, referred to the main CH_2 signal as

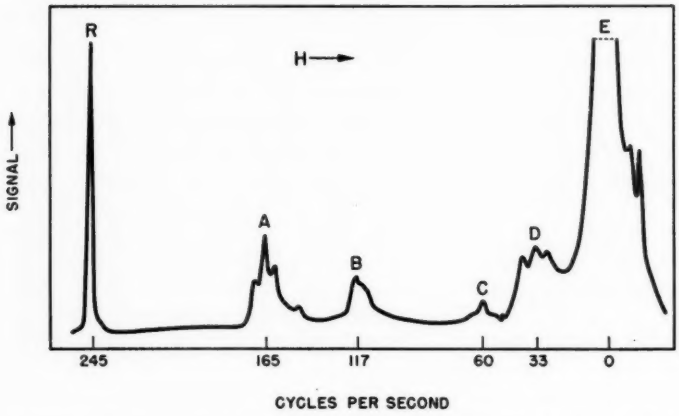


FIG. 3. Proton magnetic resonance spectra of okra seed oil (Perkins variety). Signals are assigned as follows: *A*, double bond; *B*, glyceryl residue; *C*, epoxy group; *D*, methylene groups adjacent to double bond and to carbonyl; *E*, all other CH_2 groups; *R*, chloroform reference signal.

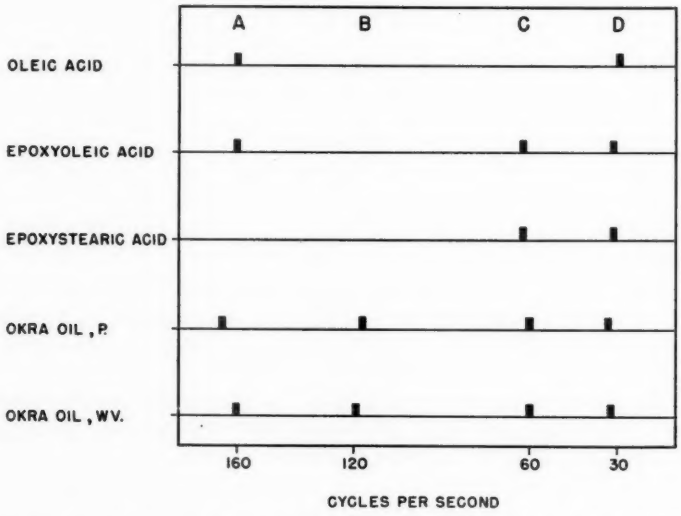


FIG. 4. Significant bands observed in the proton resonance spectra of okra seed oil and comparison compounds. Signals are assigned as in Fig. 3. Differences in intensities are not indicated. The signal of the acid proton is not shown.

zero, is attributed to the epoxy group. It is clearly evident in the spectrum of okra seed oil, thus confirming the presence of the epoxy group in the oil.

In contrast, coconut oil gave a negligible signal in this region. The seed of *Vernonia colorata* gave a strong signal at 66 c.p.s. This oil was found by chemical analysis to have a high content of epoxyoleic acid (unpublished data), resembling *Vernonia anthelmintica* in this respect.

Halphen Test

Okra seed oil is known to give a positive red color in the Halphen test and this was confirmed with both samples used in the present study. In order to determine whether the epoxy group affects the Halphen reagent (CS_2), several substances were tested. 12,13-Epoxyoleic acid, prepared from linoleic acid, gave a light brown color, not regarded as a positive reaction. Epoxystearic and diepoxystearic acids produced a yellow color, while 12,13-dihydroxyoleic acid gave no color. The oil of *Vernonia colorata* gave only a light yellow color with the Halphen reagent.

Composition of the Total Fatty Acids

The yield of dihydroxyoleic acid, after one recrystallization, was about 2.5% of the weight of the oil. (The White Velvet variety gave a larger yield, 3.8%.) If its precursor is epoxyoleic acid, as believed, the content of epoxyoleic acid in the Perkins variety is estimated to be about 3% of the total fatty acids of the oil. By calculation from the epoxy value of the whole oil, the epoxyoleic acid content is 3.9%.

The proportions of the other acids, including only those actually identified, were calculated from the distillation and analytical data and are shown in Table IV.

No dihydroxyoleic acid was obtained from the whole oil by the solvent partition procedure when the acetylation step was omitted.

TABLE IV

ESTIMATED FATTY ACID COMPOSITION
(Per cent of total fatty acids)

Acid	%
9-Hexadecenoic	0.6
Palmitic	29
Linoleic	39
Oleic	23
Stearic	2
12,13-Epoxyoleic	3
Arachidic	<1
Undetermined	3

DISCUSSION

Although a small amount of a C_{20} acid (arachidic) was found in our sample of okra seed, we did not detect any eicosenoic acid. It is judged that C_{20} acids make up a very minor proportion of the total glycerides. The unusual acid detected by Clopton and co-workers (3) was very probably epoxyoleic acid or reaction products thereof. As already known (5), the oxirane ring in this acid is quite reactive and is opened either during saponification of the glycerides or during esterification of the acids. Thus the distilled methyl ester fractions contain esters of hydroxy or methoxy acids having a chain length of 18 carbon atoms. These distill somewhat higher than the esters of ordinary C_{18} acids

and were observed during our distillation 1. Gunstone's procedure converts the epoxyoleic acid to dihydroxyoleic acid which is readily removed by solvent partition and so does not appear in the distillation.

The identity of dihydroxyoleic acid obtained in the present work from okra oil has been fully established by oxidative scission and by comparison with the same acid obtained by Gunstone and Bharucha from *Vernonia anthelmintica* (5) and *Cephalocroton cordofanus* (2). Although the reactivity of the epoxy group has prevented the isolation of epoxyoleic acid itself from these oils, there can be little doubt that they contain epoxyoleic acid as glyceride. Quantitative determination of the oxirane content of the oils agrees with the amounts of dihydroxyoleic acid isolated. In the present work, further evidence has been obtained by proton magnetic resonance spectra. Oils that yield dihydroxyoleic acid (after acetylation) exhibit a proton resonance signal corresponding to that given by the epoxy group of pure epoxyoleic and epoxystearic acids (Fig. 4).

This is the first instance to be reported of epoxyoleic acid occurring in a seed oil of the Malvaceae family. The amount is small (3%) in comparison with the proportion found in *Vernonia* and *Cephalocroton* oils (about 70%).

Although okra oil gives the Halphen test, neither epoxyoleic nor dihydroxyoleic acids give the test. Presumably, therefore, the acid responsible for the Halphen test is present in okra oil in addition to the other acids so far reported. The "Halphen" acid, believed to contain a three-carbon ring, has been found in three species of Malvaceae (9). It seems doubtful whether there is any relation between the Halphen acid and epoxyoleic acid.

In addition to the acids already known to occur in okra oil, the present work has demonstrated that 12,13-epoxyoleic and 9-hexadecenoic acids are present. Myristic acid was not detected in our sample, nor was eicosenoic acid. It is concluded that the amounts of these two acids, if present, were not more than 1% of the total. Singh and Dutt (10) reported 0.8% of myristic acid.

The estimated proportions of the major acids, palmitic, oleic, and linoleic, confirm the more recent analyses (4, 6).

ACKNOWLEDGMENTS

The authors are indebted to H. J. Bernstein and J. R. Nicholson for the nuclear magnetic resonance spectra, to A. K. Light for the ultraviolet absorption spectra, and to H. Seguin for the C and H analysis.

REFERENCES

1. Bharucha, K. E. and Gunstone, F. D. J. Chem. Soc. 1611 (1956).
2. Bharucha, K. E. and Gunstone, F. D. J. Sci. Food Agr. **7**, 606 (1956).
3. Clopton, J. R., Roberts, A., and Jeskey, H. A. J. Am. Oil Chemists' Soc. **25**, 401 (1948).
4. Crossley, A. and Hilditch, T. P. J. Sci. Food Agr. **2**, 251 (1951).
5. Gunstone, F. D. J. Chem. Soc. 1611 (1954).
6. Hussain, S. A. and Dollear, F. G. J. Am. Oil Chemists' Soc. **27**, 295 (1950).
7. Jamieson, G. S. and Baughman, W. F. J. Am. Chem. Soc. **42**, 166 (1920).
8. Lemieux, R. U. and Rudloff, E. von. Can. J. Chem. **33**, 1701 (1955).
9. Shenstone, F. S. and Vickery, J. R. Nature, **177**, 94 (1956).
10. Singh, R. and Dutt, S. Indian Soap J. **13**, 99 (1947).
11. Swern, D., Findley, T. W., Billen, G. N., and Scanlan, J. T. Anal. Chem. **19**, 414 (1947).

THE CONFIGURATION OF GLYCOSIDIC LINKAGES IN OLIGOSACCHARIDES

IV. FURTHER DEGRADATIONS OF REDUCING DISACCHARIDES TO 2-*O*-GLYCOSYL-GLYCEROLS[1]

A. J. CHARLSON,[2] P. A. J. GORIN,[2] AND A. S. PERLIN

ABSTRACT

The configuration of glycosidic linkages in a number of reducing disaccharides has been determined by converting each compound to the corresponding 2-*O*-glycosyl-glycerol, the configuration of which was readily established. Compounds examined include 3-*O*-β-D-galactopyranosyl-D-galactose, 3-*O*-α-D-galactopyranosyl-L-arabinose, 3-*O*-β-D-arabopyranosyl-L-arabinose, 2-*O*-β-D-xylopyranosyl-L-arabinose, 3-*O*-α-D-xylopyranosyl-L-arabinose, and 4-*O*-β-D-xylopyranosyl-D-xylose, all of which were derived by partial hydrolysis of polysaccharides. 2-*O*-α-L-Arabopyranosyl-glycerol has been synthesized via the Koenigs-Knorr condensation.

A previous paper in this series (3) describes a novel method for determining the configuration of glycosidic linkages in reducing disaccharides containing a hexopyranose non-reducing end-unit. According to this method the reducing end-unit of the disaccharide is degraded selectively by successive lead tetraacetate oxidations and sodium borohydride reductions to a 2-*O*-substituted glycerol. The resulting 2-*O*-glycopyranosyl-glycerol is one of two possible anomeric glycosides the configurations of which are designated by Hudson's nomenclature (9) according to their relative specific rotations.

Studies on the oxidation of reducing disaccharides by lead tetraacetate (4) indicate that many kinds of disaccharides can be degraded to give 2-*O*-glycosyl-glycerols. With the current paper the method has now been applied to each type of disaccharide represented in Fig. 1; the 1,2- and 1,4-aldohexose disaccharides were considered earlier (3). Most of the disaccharides examined had been isolated by other workers through partial hydrolysis of polysaccharides, but many had only tentatively been characterized. Included in the study are several pentopyranosyl disaccharides, and these have furnished three of the four possible 2-*O*-L-arabopyranosyl- and 2-*O*-D-xylopyranosyl-glycerols necessary as reference compounds. The fourth of these was synthesized.

Degradation of the various compounds is summarized by the series of reactions listed below, in which lead tetraacetate is represented by "O" and sodium borohydride by "H". Non-crystalline products were characterized as derived benzoates.

A. 3-*O*-β-L-Arabopyranosyl-L-arabinose (11), from a partial hydrolyzate of larch ϵ-galactan

$$\xrightarrow[(2)\ H]{(1)\ O} \text{2-}O\text{-}\beta\text{-L-arabopyranosyl-L-erythritol (hexabenzoate)}$$

$$\xrightarrow[(2)\ H]{(1)\ O} \text{2-}O\text{-}\beta\text{-L-arabopyranosyl-glycerol.}$$

[1]*Manuscript received January 3, 1957.*
Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan.
Issued as N.R.C. No. 4260 and as Paper No. 241 on the Uses of Plant Products.
[2]*National Research Council of Canada Postdoctorate Fellow, 1955–56.*

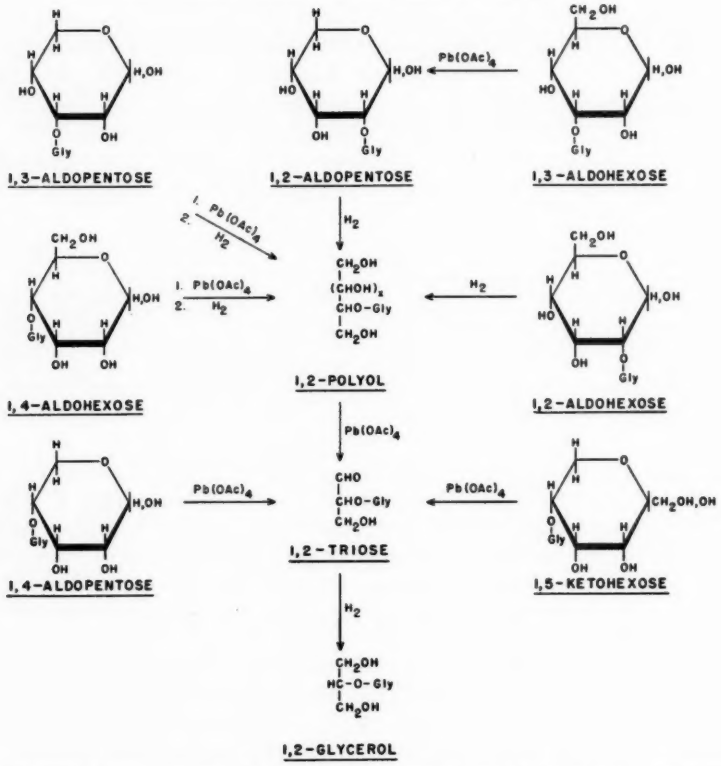


FIG. 1. A schematic representation of the reaction sequence used for degradation of reducing disaccharides to 2-*O*-glycosyl-glycerols. Non-reducing end-units of the disaccharides are denoted by "Gly".

B. 3-*O*-α-D-Xylopyranosyl-L-arabinose (1), from an autohydrolyzate of golden apple gum

$$\xrightarrow[(2)\ H]{(1)\ O}$$ 2-*O*-α-D-xylopyranosyl-L-erythritol (hexabenzoate)

$$\xrightarrow[(2)\ H]{(1)\ O}$$ 2-*O*-α-D-xylopyranosyl-glycerol (pentabenzoate).

C. 4-*O*-β-D-Xylopyranosyl-D-xylose (22) from a partial hydrolysis of wheat flour pentosan (14)

$$\xrightarrow[(2)\ H]{(1)\ 2O}$$ 2-*O*-β-D-xylopyranosyl-glycerol (pentabenzoate).

D. 2-*O*-β-D-Xylopyranosyl-L-arabinose (21) from a partial hydrolyzate of hemicellulose-B of corn cobs

$$\xrightarrow{(1)\ H}$$ 2-*O*-β-D-xylopyranosyl-L-arabitol

$$\xrightarrow[(2)\ H]{(1)\ 2O}$$ 2-*O*-β-D-xylopyranosyl-glycerol (pentabenzoate).

E. 3-*O*-α-D-Galactopyranosyl-L-arabinose (17), from a partial hydrolyzate of gum acacia

$$\xrightarrow[(2)\ H]{(1)\ O}$$ 2-*O*-α-D-galactopyranosyl-L-erythritol

$$\xrightarrow[(2)\ H]{(1)\ O}$$ 2-*O*-α-D-galactopyranosyl-glycerol.

F. 3-*O*-β-D-Galactopyranosyl-D-galactose (6, 17), from a partial hydrolyzate of gum acacia

$$\xrightarrow[(2)\ H]{(1)\ O}$$ 4-*O*-β-D-galactopyranosyl-D-arabitol (2-*O*-β-D-galactopyranosyl-D-lyxitol)

$$\xrightarrow[(2)\ H]{(1)\ 2O}$$ 2-*O*-β-D-galactopyranosyl-glycerol (hexabenzoate).

G. 5-*O*-α-D-Glucopyranosyl-D-fructose (leucrose) (19), from sucrose by enzymic synthesis with *Leuconostoc mesenteroides*

$$\xrightarrow[(2)\ H]{(1)\ 2O}$$ 2-*O*-α-D-glucopyranosyl-glycerol (hexabenzoate).

2-*O*-α-L-Arabopyranosyl-glycerol was synthesized in the following way: 2,3,4-tri-*O*-acetyl-β-L-arabopyranosyl-bromide was condensed with 1,3-*O*-benzylidene-glycerol using the procedure of Carter (2). The crystalline (1′,3′-*O*-benzylidene)-2-*O*-α-L-arabopyranosyl-glycerol thus formed gave a crystalline tribenzoate. Removal of the benzylidene group by catalytic hydrogenolysis (18) afforded sirupy 2-*O*-α-L-arabopyranosyl-glycerol, which was characterized by conversion to its pentabenzoate. The properties of this and other new glycosyl-glycerols and of their respective benzoate derivatives are listed in Table I. The majority of these benzoates appear to be amorphous. However, they have good melting points, give correct analyses, and are readily distinguishable by their specific rotations and infrared absorption spectra.

The results of the present and earlier studies in this series on determining configuration (3, 5) generally agree with those obtained previously by other workers for the same disaccharides. The sole exception is 2-*O*-β-D-xylopyranosyl-L-arabinose, which originally was thought to possess the α-configuration (20). For most of these disaccharides the assignment of configuration had been made by reference to the equilibrium specific rotations of the compounds. This practice has developed as an extension of Hudson's Rules of Isorotation (10), but its correctness has not been rigidly established, chiefly because the rotatory contribution of the reducing end-unit in the equilibrium mixture is not readily assessed. The current results, therefore, characterize more definitively those compounds examined for which configurations had been assigned previously from equilibrium rotation values. Moreover, because of the generally good agreement between the two methods, they provide support for the validity of the polarimetric method.

It appears likely that the configuration of yet other types of reducing disaccharides can be determined by the present procedure. For example, the reducing end-unit of an aldobiuronic acid may be degraded (4) and the carboxyl group of the uronic acid residue reduced to a carbinol group, giving a 2-*O*-glycosyl-glycerol. When glycosyl-glycerols required as reference compounds are not obtainable, e.g., when the related disaccharides

TABLE I

PROPERTIES OF 2-*O*-GLYCOSYL-GLYCEROLS AND DERIVATIVES

Compound	Melting point (° C.)	$[\alpha]_D^{27}$*	$[M]_D^{27}$	2B	
				Found†	Calculated‡
2-*O*-α-L-Arabopyranosyl-glycerol	Sirup	+5°	+1120		
2-*O*-α-L-Arabopyranosyl-glycerol pentabenzoate	53–57	+93°		+46820	+43140
2-*O*-β-L-Arabopyranosyl-glycerol	154–155	+204°	+45700		
2-*O*-β-L-Arabopyranosyl-glycerol pentabenzoate	48–50	+164°			
2-*O*-α-D-Xylopyranosyl-glycerol	Sirup	+95°	+21280		
2-*O*-α-D-Xylopyranosyl-glycerol pentabenzoate	51–55	+51°		+12990	+14510
2-*O*-β-D-Xylopyranosyl-glycerol	Sirup	−37°	−8290		
2-*O*-β-D-Xylopyranosyl-glycerol pentabenzoate	51–53	−36°			
2-*O*-α-D-Galactopyranosyl-glycerol hexabenzoate	156–157	+120°			
2-*O*-β-D-Galactopyranosyl-glycerol hexabenzoate	61–63	+25°			

**Of the glycosyl-glycerols, in water; of the derivatives, in 2,4-lutidine.*
†*Sum of the molecular rotations* $[M]_D$ *for the α- and β-glycosyl-glycerols; temperature 27° C.*
‡*Sum of the molecular rotations* $[M]_D$ *for the corresponding α- and β-methyl-glycosides; temperature 20° C. Values taken from Ref. 15.*

are unknown, at least one of the anomers may frequently be synthesized. This procedure is likely to prove more simple than synthesis of the disaccharide itself since it involves a condensation with readily available 1,3-disubstituted glycerols, rather than with possibly a rare sugar derivative having one particular hydroxyl group free.

EXPERIMENTAL

Optical rotations were measured at 27° C. Sugars produced by hydrolysis of disaccharides were characterized by paper chromatography. Chromatograms were prepared using Whatman No. 1 filter paper and *n*-butanol–ethanol–water (40: 11: 19, v/v) as solvent. Non-reducing and reducing sugars were detected with ammoniacal silver nitrate (13) and *p*-anisidine hydrochloride sprays (8), respectively. Solutions were concentrated at 40° C. under reduced pressure. Infrared absorption spectra were prepared by the potassium bromide window technique (16).

Disaccharides obtained by partial hydrolysis of polysaccharides were isolated by chromatographic methods. In a typical preparation, wheat flour pentosan (14) (50 g.) was hydrolyzed with 0.1 *N* sulphuric acid (250 ml.) for 5 hours on the steam bath. The products were fractionated on a charcoal column, mono-, di-, and tri-saccharides being separated with varying concentrations of ethanol in water (20). The disaccharide fraction was refractionated on a cellulose column (7) using *n*-butanol half saturated with water as solvent, and the material corresponding to xylobiose (1.0 g.) was recovered. Recrystallized from aqueous methanol it had m.p. 183–185° C., undepressed on admixture with authentic 4-*O*-D-xylopyranosyl-D-xylose.

Some of the disaccharides were sirups, in accordance with the description of these products by other workers. As a check on the isolation procedure, therefore, the component sugars of the compounds were characterized after hydrolysis, and linkage positions determined by lead tetraacetate oxidation (4).

For the sake of brevity, the techniques used in lead tetraacetate oxidations and sodium borohydride reductions are described in full only the first time they are reported. Benzoates were recrystallized from ethanol at −30° C.

2-O-β-L-Arabopyranosyl-glycerol from 3-O-β-L-Arabopyranosyl-L-arabinose

Sirupy 3-*O*-β-L-arabopyranosyl-L-arabinose (718 mg.) was dissolved in water (2 ml.), and acetic acid (150 ml.) was then added. The solution was treated with lead tetraacetate (1.2 g., 1.1 molar equivalents) and after 10 minutes the divalent lead was removed from solution by addition of 10% oxalic acid in acetic acid. The suspension was stirred for 0.5 hour, then filtered, and the filtrate was evaporated to dryness. The material obtained was dissolved in water (50 ml.) and deionized with a mixture of Amberlite IR-120 and Dowex-1 resin. Sodium borohydride (0.5 g.) was then added and after 15 minutes excess reagent was decomposed with acetic acid. The solution was treated with Amberlite IR-120 resin and evaporated to a crust, which was dissolved in methanol. The solution was evaporated in order to remove the excess boric acid. This procedure was repeated three times to give sirupy 2-*O*-β-L-arabopyranosyl-L-erythritol (415 mg.) with $[\alpha]_D$ +135° (*c*, 1.70, water).

The erythritol derivative (22 mg.) was benzoylated (procedure described under "2-*O*-α-L-arabopyranosyl-glycerol"). Two recrystallizations of the product from ethanol gave the hexabenzoate as white needles (41 mg.) with m.p. 131–134° C. and $[\alpha]_D$ +114° (*c*, 1.0, 2,4-lutidine). Calculated for $C_{51}H_{42}O_{14}$: C, 69.69%; H, 4.82%. Found, C, 70.03%; H, 4.84%.

The arabopyranosyl-erythritol (304 mg.) was oxidized with lead tetraacetate (590 mg.) in acetic acid (60 ml.) containing water (1 ml.). After the oxidation was completed the reaction mixture was worked up and the product reduced in the usual way with sodium borohydride. A mixture of compounds was produced and these were fractionated on a cellulose column using *n*-butanol half saturated with water as the mobile phase. The 2-*O*-β-L-arabopyranosyl-glycerol (83 mg.) crystallized and was twice recrystallized from ethanol. The product (64 mg.) had m.p. 154–155° C. and $[\alpha]_D$ +204° (*c*, 1.2, water). Calculated for $C_8H_{16}O_7$: C, 42.85%; H, 7.19%. Found: C, 43.04%; H, 7.20%. Hydrolysis of the material produced arabinose and glycerol. The infrared absorption spectrum of the compound differed from that of its α-anomer.

The glycerol derivative was converted to the pentabenzoate, which had m.p. 48–50° C. and $[\alpha]_D$ +164° (*c*, 0.8, 2,4-lutidine). Calculated for $C_{43}H_{36}O_{12}$: C, 69.35%; H, 4.87%. Found: C, 69.47%; H, 4.43%.

2-O-α-D-Xylopyranosyl-glycerol from 3-O-α-D-Xylopyranosyl-L-arabinose

Sirupy 3-*O*-α-D-xylopyranosyl-L-arabinose (452 mg.) was dissolved in water (2 ml.), and acetic acid (100 ml.) was added followed by lead tetraacetate (990 mg.). After 15 minutes the reaction mixture was worked up as described above and the product was reduced with sodium borohydride to give sirupy 2-*O*-α-D-xylopyranosyl-L-erythritol (338 mg.), which had $[\alpha]_D$ +91° (*c*, 0.9, water).

The erythritol derivative (42 mg.) was benzoylated and the product recrystallized three times from ethanol. It (60 mg.) had m.p. 64–67° C. and $[\alpha]_D$ +98° (*c*, 1.1, 2,4-lutidine). Calculated for $C_{51}H_{42}O_{14}$: C_6H_5CO, 71.7%. Found: C_6H_5CO, 71.5%.

2-*O*-α-D-Xylopyranosyl-L-erythritol (209 mg.) was oxidized with lead tetraacetate (400 mg.) in acetic acid (75 ml.) containing water (1.5 ml.). After the oxidation was completed the reaction mixture was worked up and the product reduced with aqueous

sodium borohydride. The resulting 2-*O*-α-D-xylopyranosyl-glycerol was contaminated with a little of the erythritol derivative and the mixture was fractionated on a cellulose column using *n*-butanol, one-third saturated with water, as solvent. The purified sirup had $[\alpha]_D$ +95° (*c*, 1.5, water) and gave xylose and glycerol on hydrolysis.

The glycerol derivative (61 mg.) was converted to its pentabenzoate, three recrystallizations from ethanol yielding a product with m.p. 51–55° C. and $[\alpha]_D$ +51° (*c*, 1.1, 2,4-lutidine). Calculated for $C_{43}H_{36}O_{12}$: C_6H_5CO, 70.6%. Found: C_6H_5CO, 70.9%.

2-O-β-D-Xylopyranosyl-glycerol from 4-O-β-D-Xylopyranosyl-D-xylose

The xylobiose (428 mg.) was dissolved in water (2 ml.), and acetic acid (100 ml.) and lead tetraacetate (1.35 g.) added. After 3 hours the 2-*O*-β-D-xylopyranosyl-D-glyceraldehyde was isolated and converted by sodium borohydride reduction to sirupy 2-*O*-β-D-xylopyranosyl-glycerol (200 mg.) with $[\alpha]_D$ −37° (*c*, 1.1, water). Hydrolysis of the material gave xylose and glycerol.

The glycerol derivative (90 mg.) was benzoylated to give the pentabenzoate, which after three recrystallizations from ethanol had m.p. 51–53° C. and $[\alpha]_D$ −36° (*c*, 0.9, 2,4-lutidine). Yield 33 mg. Calculated for $C_{43}H_{36}O_{12}$: C, 69.35%; H, 4.87%. Found: C, 69.62%; H, 4.83%. The infrared absorption spectrum differed from that of the α-anomer.

2-O-β-D-Xylopyranosyl-glycerol from 2-O-β-D-Xylopyranosyl-L-arabinose

Sirupy 2-*O*-β-D-xylopyranosyl-L-arabinose (465 mg.) was reduced to crystalline 2-*O*-β-D-xylopyranosyl-L-arabitol using sodium borohydride. The product was recrystallized twice from methanol–ethanol and had m.p. 185–187° C. and $[\alpha]_D$ −33° (*c*, 1.2, water). Yield 235 mg. Calculated for $C_{10}H_{20}O_9$: C, 42.25%; H, 7.09%. Found: C, 42.45%; H, 7.15%.

The arabitol derivative (152 mg.) was oxidized in water (0.4 ml.) and acetic acid (40 ml.) with lead tetraacetate (0.59 g.) for 18 hours. The resulting glyceraldehyde derivative was reduced with sodium borohydride to sirupy 2-*O*-β-D-xylopyranosyl-glycerol (135 mg.) having $[\alpha]_D$ −30° (*c*, 1.3, water). The infrared absorption spectrum was identical with that of an authentic specimen. The material gave xylose and glycerol on hydrolysis.

The glycerol derivative (72 mg.) was converted to its pentabenzoate, which after three recrystallizations from ethanol had m.p. 51–53° C., undepressed on admixture with an authentic specimen of 2-*O*-β-D-xylopyranosyl-glycerol pentabenzoate; $[\alpha]_D$ −35° (*c*, 1.2, 2,4-lutidine). Yield 41 mg. Calculated for $C_{43}H_{36}O_{12}$: C, 69.35%; H, 4.87%. Found: C, 69.67%; H, 4.50%. The material had an infrared absorption spectrum identical with that of authentic 2-*O*-β-D-xylopyranosyl-glycerol pentabenzoate and different from that of the α-anomer.

2-O-α-D-Galactopyranosyl-glycerol from 3-O-α-D-Galactopyranosyl-L-arabinose

3-*O*-α-D-Galactopyranosyl-L-arabinose (1.84 g.) was oxidized with lead tetraacetate (2.5 g.) for 10 minutes in acetic acid (250 ml.) containing water (4 ml.). The reaction product was converted by sodium borohydride reduction to 2-*O*-α-D-galactopyranosyl-L-erythritol, which on recrystallization from aqueous ethanol had m.p. 156–158° C. and $[\alpha]_D^{\bullet}$ +145° (*c*, 1.0, water). Yield 1.0 g. Calculated for $C_{10}H_{20}O_9$: C, 42.25%; H, 7.09%. Found: C, 42.16%; H, 7.11%.

The erythritol derivative (560 mg.) was oxidized for 3 hours with lead tetraacetate (860 mg.) in acetic acid (100 ml.) containing water (2 ml.). The reaction mixture was

worked up and the product reduced with aqueous sodium borohydride to give a mixture of unchanged material and 2-*O*-α-D-galactopyranosyl-glycerol. These were separated on a cellulose column using *n*-butanol half saturated with water as solvent. The gylcosyl-glycerol (250 mg.) was recrystallized from ethanol and had m.p. 131–132° C., undepressed on admixture with an authentic specimen of 2-*O*-α-D-galactopyranosyl-glycerol (12); $[\alpha]_D$ +164° (*c*, 0.7, water). Calculated for $C_9H_{18}O_8$: C, 42.52%; H, 7.14%. Found: C, 42.55%; H, 7.17%. The infrared absorption spectra of the product and of the authentic α-anomer were identical.

2-O-α- and 2-O-β-D-Galactopyranosyl-glycerol Hexabenzoates

2-*O*-α-D-Galactopyranosyl-glycerol (46 mg.) was benzoylated to give its hexabenzoate (76 mg.) having m.p. 156–157° C. and $[\alpha]_D$ +120° (*c*, 1.4, 2,4-lutidine) after two recrystallizations from ethanol. Calculated for $C_{51}H_{42}O_{14}$: C, 69.69%; H, 4.82%. Found: C, 69.24%; H, 4.73%.

The β-anomer (46 mg.) was benzoylated yielding, after four precipitations from ethanol, an amorphous powder (53 mg.) which had m.p. 61–63° C. and $[\alpha]_D$ +25° (*c*, 0.6, 2,4-lutidine). Calculated for $C_{51}H_{42}O_{14}$: C, 69.69%; H, 4.82%. Found: C, 69.20%; H, 5.37%. The two anomeric hexabenzoates had readily distinguishable infrared absorption spectra.

2-O-β-D-Galactopyranosyl-glycerol from 3-O-β-D-Galactopyranosyl-D-galactose

3-*O*-β-D-Galactopyranosyl-D-galactose (500 mg.) was dissolved in acetic acid (100 ml.) containing water (1 ml.) and oxidized with lead tetraacetate (900 mg.). After 20 minutes the reaction mixture was worked up and reduced with sodium borohydride to give 4-*O*-β-D-galactopyranosyl-D-arabitol (2-*O*-β-D-galactopyranosyl-D-lyxitol (390 mg.), which was recrystallized twice from ethanol to give a material with m.p. 177–179° C. and $[\alpha]_D$ −7° (*c*, 0.6, water). Calculated for $C_{11}H_{22}O_{10}$: C, 42.03%; H, 7.06%. Found: C, 41.99%; H, 7.06%.

The disaccharide (310 mg.), thus obtained, was oxidized with lead tetraacetate (860 mg.) for 2 hours in acetic acid (50 ml.) containing water (0.8 ml.). The product was reduced with aqueous sodium borohydride to give a mixture which contained 2-*O*-β-D-galacto-pyranosyl-glycerol. This was fractionated on a cellulose column using *n*-butanol half saturated with water as the mobile phase. The D-galactosyl-glycerol (100 mg.) could not be induced to crystallize. Therefore a portion (41 mg.) was converted to its hexa-benzoate, which after two successive precipitations from ethanol had m.p. 56–59° C., undepressed on admixture with authentic 2-*O*-β-D-galactopyranosyl-glycerol hexaben-zoate, and $[\alpha]_D$ +36° (*c*, 0.7, 2,4-lutidine). Calculated for $C_{51}H_{42}O_{14}$: C, 69.69%; H, 4.82%. Found: C, 69.53%; H, 4.83%. The infrared absorption spectrum was identical with that of the β-galactose hexabenzoate derivative and differed from that of the α-anomer.

2-O-α-D-Glucopyranosyl-glycerol from 5-O-α-D-Glucopyranosyl-D-fructose

5-*O*-α-D-Glucopyranosyl-D-fructose (225 mg.) was oxidized with lead tetraacetate (580 mg.) in acetic acid (50 ml.) containing water (1 ml.). After 3 hours' reaction time the product was reduced using sodium borohydride to give a mixture of materials from which 2-*O*-α-D-glucopyranosyl-glycerol (85 mg.) was isolated by chromatography on a cellulose column using *n*-butanol half saturated with water as solvent. It had $[\alpha]_D$ +119° (*c*, 0.7, water), an infrared absorption spectrum identical with that of the α-anomer but

different from that of the β-anomer, and gave glucose and glycerol on acid hydrolysis.

The glycerol derivative was converted to its hexabenzoate, which was recrystallized twice from ethanol. It had m.p. 137–138° C., unchanged on admixture with authentic 2-*O*-α-D-glucopyranosyl-glycerol hexabenzoate, and $[\alpha]_D$ +94° (*c*, 0.8, $CHCl_3$); it gave an infrared absorption spectrum identical with that of 2-*O*-α-D-glucopyranosyl-glycerol hexabenzoate, but unlike the β-anomer. Calculated for $C_{51}H_{42}O_{14}$: C, 69.69%; H, 4.82%. Found: C, 69.82%; H, 4.80%.

2-O-α-L-Arabopyranosyl-glycerol

A mixture of tri-*O*-acetyl-β-L-arabopyranosyl-bromide (8.4 g.), 1,3-*O*-benzylidene-glycerol (7.5 g.), silver carbonate (20 g.), and Drierite (50 g.) in benzene (125 ml.) was shaken for 3 hours at 55° C. It was then refluxed for 15 minutes, the solution was filtered, and chloroform (200 ml.) was then added to the filtrate followed by sodium methoxide (0.5 g.) in methanol (20 ml.). After 18 hours at room temperature the solution was evaporated to a sirup which was fractionated on a cellulose column. Benzene eluted unchanged 1,3-*O*-benzylidene-glycerol, and benzene–ethanol–water (3:1: trace, v/v) eluted (1′,3′-*O*-benzylidene)-2-*O*-α-L-arabopyranosyl-glycerol (3.1 g.). The product crystallized and was recrystallized twice from methanol–ether, m.p. 121–122° C. and $[\alpha]_D$ +4° (*c*, 1.1, ethanol). Calculated for $C_{15}H_{20}O_7$: C, 57.68%; H, 6.40%. Found: C, 57.73%; H, 6.48%.

The product (46 mg.) was dissolved in chloroform (0.30 ml.) containing pyridine (0.20 ml.) and benzoyl chloride (0.12 ml.). After 18 hours the reaction mixture was dissolved in chloroform to which a little methanol was added, and the solution was washed successively with 1% sulphuric acid, aqueous sodium bicarbonate, and water, then dried with magnesium sulphate, filtered, and evaporated to a sirup which crystallized. Three recrystallizations from ethanol gave a product softening at 160–165° C. and melting at 170–172° C., with $[\alpha]_D$ +114° (*c*, 0.9, 2,4-lutidine). Calculated for $C_{36}H_{32}O_{10}$: C, 69.22%; H, 5.16%. Found: C, 69.47%; H, 5.18%.

The 1,3-benzylidene-L-arabinosyl-glycerol (28 mg.) was dissolved in ethanol (25 ml.) and the solution was shaken with palladium oxide (80 mg.) in an atmosphere of hydrogen for 18 hours. The product, obtained as a sirup by filtration and evaporation of the solution, had $[\alpha]_D$ +5° (*c*, 1.0, water).

2-O-α-L-Arabopyranosyl-glycerol Pentabenzoate

The L-arabopyranosyl-glycerol (93 mg.) was benzoylated and the product, after three recrystallizations from ethanol, had m.p. 53–57° C. and $[\alpha]_D$ +93° (*c*, 0.9, 2,4-lutidine). Yield 61 mg. Calculated for $C_{43}H_{36}O_{12}$: C_6H_5CO, 70.6%. Found: C_6H_5CO, 70.8%.

ACKNOWLEDGMENT

The authors gratefully thank Dr. W. G. C. Forsyth, Colonial Microbiological Research Institute, Port of Spain, for a sample of golden apple gum, Dr. J. R. Nunn, University of Cape Town, for a specimen of 2-*O*-α-D-galactopyranosyl-glycerol, and Dr. Frank H. Stodola, Northern Utilization Research Branch, Peoria, for a sample of leucrose. Thanks are also due to Mr. J. A. Baignée for carbon and hydrogen microdeterminations, Mr. J. W. L. C. Christ for technical assistance, and Miss A. Epp for infrared studies.

REFERENCES

1. ANDREWS, P. and JONES, J. K. N. J. Chem. Soc. 4134 (1954).
2. CARTER, N. M. Ber. **63**, 1684 (1930).
3. CHARLSON, A. J., GORIN, P. A. J., and PERLIN, A. S. Can. J. Chem. **34**, 1811 (1956).
4. CHARLSON, A. J. and PERLIN, A. S. Can. J. Chem. **34**, 1200 (1956).
5. CHARLSON, A. J. and PERLIN, A. S. Can. J. Chem. **34**, 1804 (1956).
6. HIRST, E. L. and PERLIN, A. S. J. Chem. Soc. 2622 (1954).
7. HOUGH, L., JONES, J. K. N., and WADMAN, W. H. J. Chem. Soc. 2511 (1949).
8. HOUGH, L., JONES, J. K. N., and WADMAN, W. H. J. Chem. Soc. 1702 (1950).
9. HUDSON, C. S. J. Am. Chem. Soc. **31**, 66 (1909).
10. HUDSON, C. S. J. Am. Chem. Soc. **38**, 1566 (1916).
11. JONES, J. K. N. J. Chem. Soc. 1672 (1953).
12. NUNN, J. R. and HOLDT, M. M. VON. J. Am. Chem. Soc. **77**, 2551 (1955).
13. PARTRIDGE, S. M. Nature, **158**, 270 (1946).
14. PERLIN, A. S. Cereal Chem. **28**, 370 (1951).
15. POLARIMETRY, SACCHARIMETRY, AND THE SUGARS. Natl. Bur. Standards (U.S.), Circ. C440, Govt. Printing Office, Washington, D.C. 1942.
16. SCHIEDT, U. and REINWEIN, H. Z. Naturforsch. **7b**, 270 (1952).
17. SMITH, F. J. Chem. Soc. 744 (1939).
18. STIMMEL, B. F. and KING, C. G. J. Am. Chem. Soc. **56**, 1774 (1934).
19. STODOLA, F. H., KOEPSELL, H. J., and SHARPE, E. S. J. Am. Chem. Soc. **74**, 3202 (1952).
20. WHISTLER, R. L. and DURSO, D. F. J. Am. Chem. Soc. **72**, 677 (1950).
21. WHISTLER, R. L. and MCGILVRAY, D. I. J. Am. Chem. Soc. **77**, 1884 (1955).
22. WHISTLER, R. L. and TU, C. C. J. Am. Chem. Soc. **73**, 1389 (1951).

A RECORDING VACUUM THERMOBALANCE[1]

J. G. HOOLEY

ABSTRACT

A recording quartz spring balance is described, with which weight changes up to 1000 mg. can be followed linearly on a millivolt recorder for samples weighing up to 10 g. During the recording operation the sample may be under vacuum or pressure and may be heated or cooled. The maximum practical sensitivity is about 20 millivolts per milligram and the over-all error in reading the record is about 1 millivolt. The temperature coefficient of fused quartz springs is reported to be $-(1.25\pm0.02)\times10^{-4}$ per degree centigrade in the range 20° to 35° C.

The device could be readily adapted for heavier loads and greater weight changes by using less sensitive springs and could be used for measuring dimensional changes of solids with a maximum sensitivity of at least 2000 mv. per millimeter.

INTRODUCTION

Various recording thermobalances are described in the literature. Some use beams supported by metal wires or conventional knife edges, but operate only at atmospheric pressure. In these a continuous record of weight is supplied by an optical system (1) or by an electronic circuit (3, 5). Others use the twisting or bending of a quartz fiber to measure the weight change. These balances can be enclosed in a vacuum and can be made recording by an electronic circuit (6). They are of low capacity and very subject to external vibrations. On the other hand, they are extremely sensitive and are indispensable for the weighing of small amounts of material.

The quartz spring balance can also operate at any pressure and can handle a much heavier load than a torsion balance (6). To date, however, it has not been made recording. This article describes a recording spring balance of simple and economical construction, whose sensitivity can be easily varied. The essential parts are illustrated in Figs. 1 and 2. The quartz spring C supports the steel core E of a linear variable differential transformer F and below it the sample M, whose weight is to be followed. The transformer has one primary and two secondaries in opposition to one another. As the core moves in response to the changing weight of the sample, the net output voltage varies. This is amplified, converted to direct current, and fed into a millivolt recorder.

DETAILS OF DESIGN AND OPERATION

Spring and Circuit Adjustments

In Fig. 1, B is a piece of sheet Pyrex held in a vertical position by four projections on the inside of the enveloping tube. In the setting up of the balance, B is first suspended outside and to it is hooked a spring, a quartz fiber D, and the quartz enclosed transformer core E. The whole is lowered into the tube, which is then capped and sealed at A. The fiber L is hooked to the core and to the quartz pan M containing a weighed sample. The six levelling screws in the base of the instrument are then adjusted so that the core–fiber–pan system rides freely in space. The lateral position of the core is not critical.

The recorder is next adjusted to read 50 mv. at zero voltage, i.e. when shorted. This will later correspond to having the core in the vertical center of the transformer. Vertical

[1]*Manuscript received December 26, 1956.*
Contribution from the Department of Chemistry, University of British Columbia, Vancouver, B.C., with financial assistance from the National Research Council.

displacement from this position produces a positive or negative voltage, depending on the direction of motion. The magnitude of this voltage increases with displacement and with primary voltage. The latter must therefore be constant for a given run. It is supplied by a divider across a 6.3 volt Sola constant voltage transformer. Each potential can be used with either the 20 db. or 40 db. setting of the amplifier. The particular combination chosen is determined by the desired sensitivity. If this sensitivity is such that the subsequent weight change moves the recorder pen off the scale, it can be brought back by the scale shifter between the amplifier and the converter. This is a variable source of constant a-c. voltage from a divider across a 1:1 transformer fed by the same 6.3 volt Sola transformer that feeds the primary circuit.

The height of the transformer *F* is next adjusted so that the recorder pen is at zero or 100, depending on whether the weight change will move it up or down. This is accomplished by coarse and fine adjustments to the height of the wooden clamp holding the transformer. The length of the fiber *D* was initially chosen so that this adjustment could be made. The tube *N* is now placed in position and tubes *K* and *H* are used to control

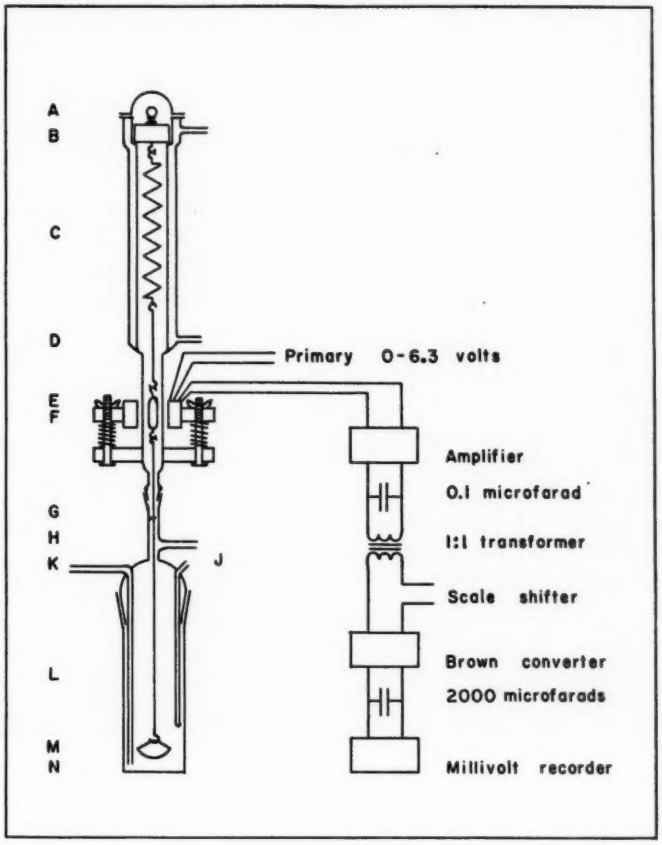


FIG. 1. The balance and the recording circuit.

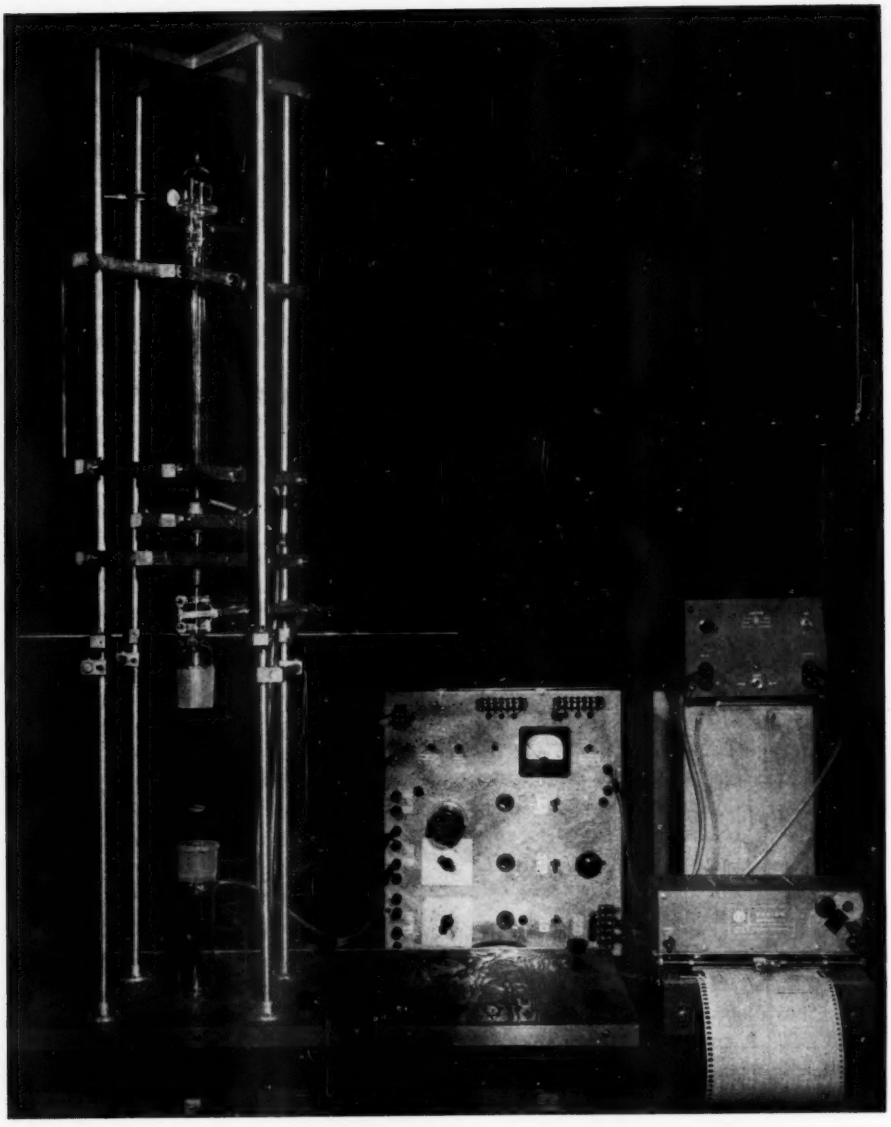


FIG. 2.

the atmosphere and its pressure. A thermocouple in tube J is used to measure the temperature. A tube furnace or cold bath may be placed around N. The millivolt record is converted to milligrams by using the predetermined sensitivity.

During the operation of the balance, the spring may begin to oscillate, in which case it may be several minutes before it has died down sufficiently so that the record is again a smooth line. This time may be reduced to a few seconds by the glass fiber G which extends across the center of the tube. The oscillating fiber L is brought into light contact with G by adjustment of the levelling screws in the base of the instrument.

Transformer-support Temperature Control

The temperature coefficient of an unfavorable support design may be as high as 1 milligram per degree. If, however, the transformer is supported as shown in Fig. 1, then the expansion of the two 7.5 cm. brass bolts equals that of the 42 cm. of Pyrex tubing between the lower clamp and the suspension B. The coefficient is thereby reduced to zero if the tube and support are at the same temperature. Actually, the thermostated spring tube is seldom at room temperature and a correction must be applied for the difference.

Spring Temperature Control

The temperature coefficient of the spring may be as high as -2 mg. per degree. Water from a thermostated bath is therefore circulated through the jacket around the spring tube. This jacket and the special transformer support are not shown in Fig. 2.

PERFORMANCE AND CALIBRATION

Reproducibility

For a constant load, sensitivity, and temperature, the record was constant over a period of 8 hours. If everything was then turned off and on again the following day, the record was the same within 1 mv. after an initial warm-up period. At sensitivities over 10 mv. per mg. the pen did follow the temperature variation of $\pm 0.05°$ C. of the water bath.

Linearity

The millivolt record was found to be a linear function of the weight added if the core displacement was less than 0.20 cm. from its central position. Within this range the deviation of the points from a straight line was always less than 1 mv. This region of linearity corresponds to a weight change of 1000 mg. for a class C_2 spring down to 250 mg. for a class E spring. This is illustrated in Fig. 3 for spring D_1 with a linear range of 400 mg.

Sensitivity

This was determined by measuring the slope of the straight line through at least six points in the plot of millivolt output against weight added. In Fig. 4 its value for spring D_1 is plotted against primary voltage as measured by a vacuum tube voltmeter. Values for the other springs were also measured and can be calculated from the sensitivities in cm. $g.^{-1}$ listed in Table I. It was shown that for a given primary voltage, the standard deviation from the average sensitivity was always less than 1%. This applied to averages of values taken at temperatures from 15° to 35° C. and for total loads from 10 to 15 g.

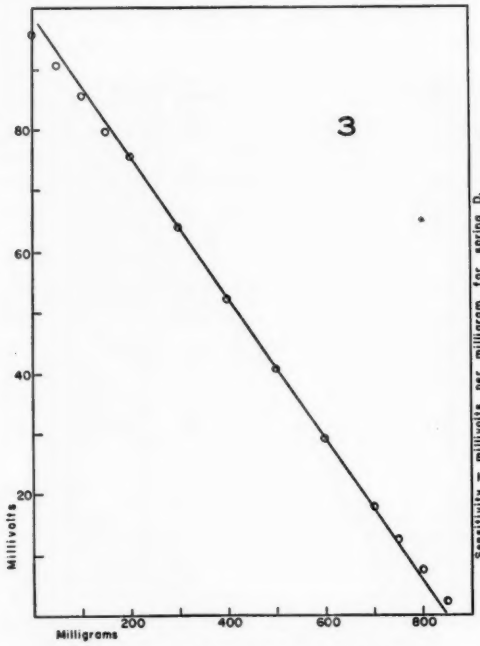


FIG. 3. The linear region for spring D_1.

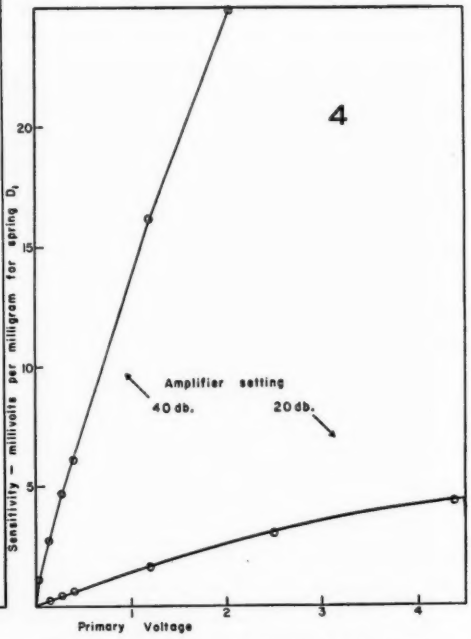


FIG. 4. Sensitivity as a function of voltage.

TABLE I

SPRING CHARACTERISTICS

Spring	Sensitivity, cm. g.$^{-1}$	Weight of spring, g.	Total load,* g.	Number of helices	Fiber diameter, cm.	Fractional temperature coefficient per °C.$\times 10^4$
C_1	0.463	1.310	11.364	61	0.0535	−1.27
			13.349			−1.28
			15.279			−1.27
C_2	0.403	0.342	16.290	27	0.0421	−1.24
D_1	1.001	1.700	11.209	95	0.0494	−1.24
			11.219			−1.23
			15.969			−1.21
D_2	1.120	1.408	11.828	89	0.0464	−1.26
			15.823			−1.23
E	1.532	1.012	11.125	88	0.0410	−1.25
			14.625			−1.24
				Average		−1.25
						±0.02
Sheft and Fried	7.30	0.84	1.696	80	0.035	−1.18
			2.088			−0.96

Includes connecting fibers and half the weight of the spring.

Sensitivities up to 50 mv. mg.$^{-1}$ were obtainable, but the record was too much affected by building vibrations and temperature variation of the spring to be useful. The upper practical limit was about 20 mv. mg.$^{-1}$ for spring D_1, which is equivalent to 5000 Å per mv.

Temperature Coefficient

(*a*) *The Transformer Support*

As mentioned above, the relative lengths of the brass bolts and the Pyrex tube above the clamp can be adjusted so that their total expansions per degree are equal. When the temperature of the whole assembly changes, there is therefore no change in the relative position of the core with respect to the transformer, neglecting for now any contribution by the spring itself. However, the jacketed spring tube and the support are usually at different temperatures. The correction to be applied was determined in two ways. In each the air around the support and up to the jacket was thermostated by passing heated air through an insulated polythene bag fitted around that section. In the first method, the temperature of the jacket was held constant and the millivolt record was noted as a function of air temperature and converted to centimeters. In the range 22° to 32° C. a coefficient of -8.5×10^{-5} cm. per ° C. was obtained. The minus sign is used to show that the core moved down relative to the transformer as the air temperature increased relative to the jacket temperature. In the second method, a silica fiber was used in place of a spring. The air temperature was held constant and the millivolt record noted as a function of jacket temperature. A coefficient of -7.7×10^{-5} cm. per ° C. was obtained. When this is corrected for the expansion of the 30 cm. silica fiber, a value of -9.2×10^{-5} cm. per ° C. is obtained for the Pyrex tube itself. In actual runs there is present a length of silica fiber D which varies from 5 to 20 cm. A uniform value of -8.5×10^{-5} cm. per ° C. was therefore used as a correction factor.

(*b*) *The Spring*

Thermometers were placed at the inlet and outlet of the water jacket. During operation these showed a temperature difference of less than 0.02° C. and a variation with time of ±0.05° C. When the temperature setting was changed, the record became constant again within 2 minutes after the new temperature was reached.

Coefficients were measured as follows: Millivolt readings were taken for several weights at four temperatures. For each temperature a straight line was drawn through the plot of voltage against added weight. The weight values at 50 mv. were then corrected for the difference between the temperatures of the transformer support and the tube jacket. These corrected weights were plotted against temperature and in all cases a straight line fitted the data to ±0.1%. The temperature coefficient and its value divided by the total load (including half the weight of the spring) is listed in Table I for five springs at various total loads. The average value is $-(1.25\pm0.02)\times10^{-4}$ per centigrade degree. The data show it to be independent of fiber diameter, the number of coils, and the diameter of the coils, which varied from 1.28 cm. for E to 1.39 cm. for C_1.

DISCUSSION

The springs used in this work limited the sample weight to a maximum of 10 g. and the weight change for a linear voltage response to 1 g. Both these maxima could easily be increased by using less sensitive springs.

The apparatus could also be used to measure dimensional changes of solids under various conditions of temperature, pressure, and atmosphere. The only requirement is that it must be possible to hook the sample into the system in place of the quartz spring. The maximum sensitivity would be at least 2000 mv. per millimeter. This is equivalent to the practical upper limit of 20 mv. $mg.^{-1}$ observed for spring D_1.

The temperature coefficient of quartz springs should be equal in magnitude and opposite in sign to the temperature coefficient of the torsional modulus of quartz (2). This latter value, as determined for quartz fibers in the range 15° to 100° C. by Horton (4), is 1.235×10^{-4} per centigrade degree. This is within the experimental error of the value predicted by the work reported in this paper.

The temperature coefficient of a quartz spring has been determined by Sheft and Fried (7) using an optical system for measurement of length. Their two values of -2×10^{-4} per centigrade degree do not include any of the weight of the spring or connecting fibers. These weights were obtained by private communication and when used to recalculate their values to conform to those in the present paper gave the coefficients listed in Table I. The two new values of -1.18 and -0.96×10^{-4} are to be compared with the value of $-1.25\pm0.02\times10^{-4}$ reported in this paper. The greater spread and the difference may well be the result of the lower sensitivity of the optical method of observation.

Theoretically, the sensitivity in units of extension per unit load is inversely proportional to the torsional modulus. The temperature coefficient of the sensitivity should therefore be -1.25×10^{-4} per centigrade degree. Hence for the 20 degree range of temperature used in this work, the sensitivity should change by 0.24%. This is less than the observed standard deviation of 1% and was therefore not detected.

APPENDIX

The main components came from the following suppliers:

Springs—Houston Technical Laboratories.

Transformer—Type 060 S-LB from Schaevitz Engineering, Camden 1, N.J.

Amplifier—Model 450 A from Hewlett-Packard Co., Palo Alto, Calif.

Converter—Brown type from Minneapolis-Honeywell.

Recorder—Model G 10 from Varian Associates of Canada, Georgetown, Ont.

ACKNOWLEDGMENT

Advice regarding the electronic circuit was kindly provided by Dr. J. Brown and Mr. W. Corfield of the Departments of Physics and Chemistry respectively.

REFERENCES

1. Duval, C. Inorganic thermogravimetric analysis. Elsevier Pub. Co., Inc., Amsterdam. 1953.
2. Ernsberger, F. M. Rev. Sci. Instr. **24**, 998 (1953).
3. Gordon, S. and Campbell, C. Anal. Chem. **28**, 124 (1956).
4. Horton, F. J. Trans. Roy. Soc. (London), A, **204**, 407 (1905).
5. McBain, J. W. and Tanner, H. G. Proc. Roy. Soc. (London), A, **125**, 579 (1929).
6. National Bureau of Standards (U.S.), Circ. 569, Fused quartz fibres, 18 (1956).
7. Sheft, I. and Fried, S. Rev. Sci. Instr. **19**, 723 (1948).

VISCOSITY OF NATURAL RUBBER SOLUTIONS AT VERY LOW RATES OF SHEAR[1]

MORTON A. GOLUB

ABSTRACT

The shear dependence of viscosity of benzene solutions of natural rubber was studied at rates of shear from about 500 down to less than 1 sec.$^{-1}$. Measurements involved following the change of pressure head with time of the various solutions flowing in a capillary, U-tube viscometer. Curvature in the plots of the logarithm of pressure head versus time indicated non-Newtonian flow. From such curves, reduced viscosity data over the above-mentioned shear range were readily derived. As a check, data over the range 100–500 sec.$^{-1}$ were also obtained with a five-bulb viscometer of the Krigbaum–Flory type, and these data overlapped those obtained with the U tube. The reduced viscosity increased very sharply with decrease in gradient, making extrapolation to the viscosity axis quite unreliable. However, a theoretical relation proposed by Bueche fitted the composite data rather well. This work furnished a nice technique for determining the zero shear reduced viscosity $(\eta_{sp}/c)_0$ without the necessity of performing an uncertain extrapolation: evaluate the parameters of the Bueche formula which best satisfies the experimental data over a fairly wide range of shear rates, and then calculate $(\eta_{sp}/c)_0$ directly.

INTRODUCTION

In a previous paper (3) describing reduced viscosity measurements on Alfin polyisoprene over the fairly wide range of shear rates 100–20,000 sec.$^{-1}$, it was shown that a theoretical relation proposed by Bueche (1) to account for the non-Newtonian flow of high polymer solutions fitted the experimental data rather well, at least up to about 12,000 sec.$^{-1}$. Beyond this point, the Bueche plot fell steadily below the experimental points. Inasmuch as his theoretical expression was derived by dropping terms which become important only at large shear rates, it appeared that the Bueche equation, when modified to incorporate some of these terms, would fit the over-all data quite well even up to about 20,000 sec.$^{-1}$. At the same time, the revised equation would have to take into consideration the ultimate asymptotic levelling-off of the reduced viscosity at very high gradients.

As recommended by Bueche and indicated in the earlier paper, there is a definite need to obtain further data at shear rates well below 100 sec.$^{-1}$ in order to ascertain the reliability of the Bueche relation in the very low shear range. Since the gradient enters to the one-half power in the equation at low shear rates, this relation implies a virtually infinite slope of the reduced viscosity – shear curve at the ordinate. Previous work (3), however, over the range 100–500 sec.$^{-1}$ showed the reduced viscosity to increase in an approximately linear fashion with decrease in rate of shear. Consequently, the exact shape of the reduced viscosity – shear plot at the smallest possible gradients deserves renewed attention.

Chang and Morawetz (2) have recently described a procedure for determining the shear dependence of viscosity at very low rates of shear. This involves following the change of pressure head with time of a liquid flowing in a capillary U-tube viscometer. Curvature in the plots of the logarithm of pressure head versus time indicates non-Newtonian behavior. By means of this procedure, measurements could be readily extended down to shear rates somewhat below 1 sec.$^{-1}$. This is about the lower practical limit of shear rate attainable in a capillary viscometer for a liquid with a viscosity of about 10 to 20 centipoises.

[1]*Manuscript received November 7, 1956.*
Contribution from the B. F. Goodrich Company Research Center, Brecksville, Ohio. Presented at the Seventh Canadian High Polymer Forum, Sarnia, Ontario, November, 1956.

In connection with attempts at investigating microstructural differences in polymers through differences of the shear effects, recent attention in these laboratories has been focussed on the natural rubber molecule. It has been observed, for example, that the shear dependence of intrinsic viscosity for the polyisoprene molecule—be it the natural all-*cis*, or the predominantly *trans* Alfin polymer—is pretty much the same for the two substances (4). For an examination of the Bueche equation at very small gradients, natural rubber was consequently selected for study. This paper reports the results obtained from reduced viscosity measurements on *Hevea* solutions at rates of shear from about 500 down to less than 1 sec.$^{-1}$, using the method of Chang and Morawetz

EXPERIMENTAL

Two U-tube viscometers were used in this work, one covering the shear range 100 down to less than 1 sec.$^{-1}$ (MV-2), the other covering the wider range 0–500 sec.$^{-1}$ (MV-4). Each arm of the former viscometer contains a 25 cm. length of precision bore capillary (radius 0.025 cm.) for a total capillary length of 50 cm. A 14 cm. length of precision bore capillary (radius 0.020 cm.) is contained in each arm of the latter viscometer. These instruments are essentially continuously varying pressure head viscometers. Identical cylindrical working volumes in the two arms of each viscometer are assured by the use of precision bore tubing above the capillaries (0.6 cm. I.D.; height 20 cm. in MV-2, 31 cm. in MV-4). To avoid having a curved capillary in the U tubes, the arms are joined at the bottom by a piece of tubing of about 6 mm. inside diameter. The over-all heights of both viscometers are about 47 cm. They were firmly supported in a vertical position in a constant temperature bath set at 25±0.01° C. The bath was an aquarium type with plate glass sides suitable for viewing the menisci with a cathetometer.

The procedure involved first adjusting the liquid levels above the capillaries so that a driving pressure of about 3–5 cm. of benzene in MV-2, or about 13–16 cm. in MV-4, was established. Then measurements were made of the levels in the viscometer arms at various time intervals, as the liquid flowed through the capillary under its own weight, until the driving pressure fell to about 2 or 3 mm. From the definition of viscosity η, it may be seen that for Newtonian flow

$$\ln h/h_0 = -(gR^4d/4a^2\eta l)t = St, \qquad [1]$$

where h and h_0 are the actual differences in liquid levels at time t and at the outset of a run respectively, d is the density of the liquid, R and l are the capillary radius and length, a is the radius of the cylindrical working volume, and g is the gravitational constant. Fig. 1 shows the linear plots of log h/h_0 vs. t for a Newtonian liquid, such as benzene, in the low shear viscometers. The good agreement between the measured slopes of the lines[2] (-3.20×10^{-3} and -2.32×10^{-3} sec.$^{-1}$ for MV-2 and MV-4, respectively) and the corresponding values for S calculated from the approximate instrumental dimensions and known constants of benzene (-3.08×10^{-3} and -2.25×10^{-3} sec.$^{-1}$) using equation [1] is evidence for the reliability of this approach. Obviously this procedure could serve as an absolute method for determining the viscosity of a Newtonian liquid.

Curvature in the log h/h_0 vs. t plot indicates non-Newtonian behavior. If S is the slope of such a plot for a solution at some particular value of h, and S_0 the slope for the solvent, then the relative viscosity of the solution at the rate of shear corresponding to the given head is simply S_0/S. By drawing tangents at a number of points in each of the experi-

[2]*Based on the corresponding natural logarithm plots.*

mental curves and evaluating S_0/S for the respective gradients, reduced viscosity data over the range of shear rates mentioned above for the two viscometers were readily derived for the various natural rubber solutions.

No kinetic energy corrections were applied to the flow data in this work since these were considered negligible under the given experimental conditions. Mock and Marshall (6) have recently discussed the kinetic energy correction for this type of viscometer.

As a further check on the reliability of the data obtained by the above method, viscosity measurements over the range 100–500 sec.$^{-1}$ were also made with a five-bulb viscometer of the Krigbaum–Flory type. The procedure followed with this viscometer has been described before (3).

The maximum rate of shear, D, at the wall was calculated by means of the relation

$$D = Rghd/2\eta l. \qquad [2]$$

The natural rubber was a sample of pale crepe, having a viscosity average molecular weight of about 2,100,000. Viscosity measurements were carried out on gel-free benzene solutions varying in concentration from about 0.05 to 0.5 g./dl. The rubber solutions contained tetramethylthiuram disulphide to the extent of 1% on polymer for stabilization.

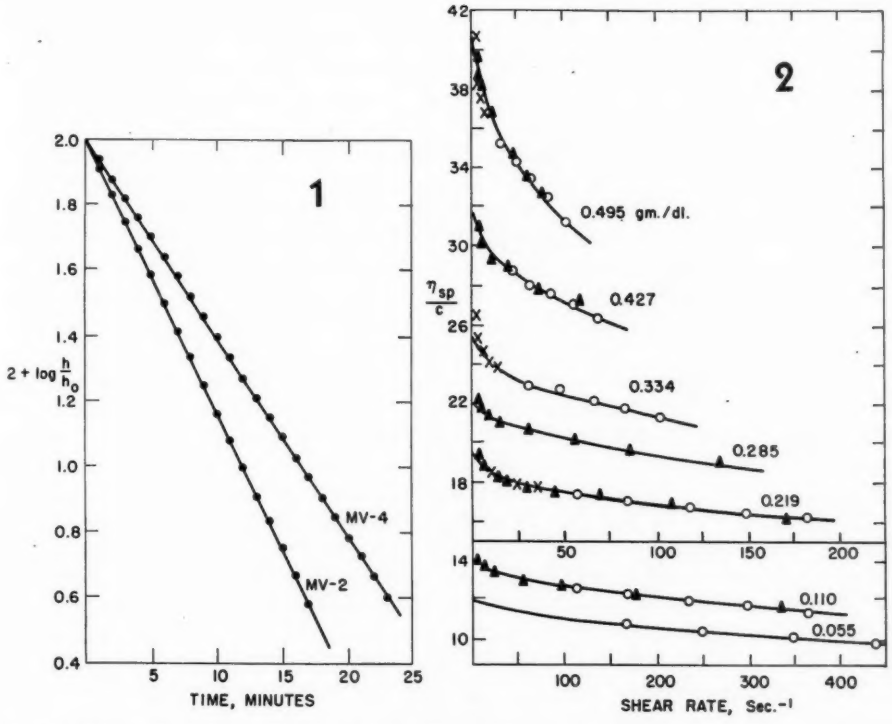


FIG. 1. Plots of log h/h_0 vs. time for a Newtonian liquid (benzene) in the two low shear viscometers.
FIG. 2. Effect of rate of shear on the reduced viscosity of natural rubber in benzene at 25° C. The various points were obtained with the five-bulb viscometer (○), and U-tube viscometers MV-2 (×) and MV-4 respectively (▲). The lines were derived from the Bueche equation.

RESULTS AND DISCUSSION

Typical flow data for various concentrations of natural rubber in benzene, obtained in the MV-2 viscometer, are shown as the points joined by the solid lines in Fig. 3. The dotted lines are tangents to the curves at the onset of flow in the U-tube viscometer. The slopes of these tangents, S, divided by that for benzene, S_0, give the relative viscosities for the several solutions corresponding to the initial hydrostatic heads (and hence mean shear gradients). These solutions were also run in the five-bulb viscometer (see Fig. 2) in which the lowest rate of shear was higher than the highest in the MV-2 viscometer. Significantly, the initial reduced viscosity values, η_{sp}/c, obtained in the latter instrument as described above are all larger than corresponding values obtained in the five-bulb viscometer, and moreover are approximately the values which would be predicted by extrapolation of the nearly linear plots of the five-bulb data. Now, in

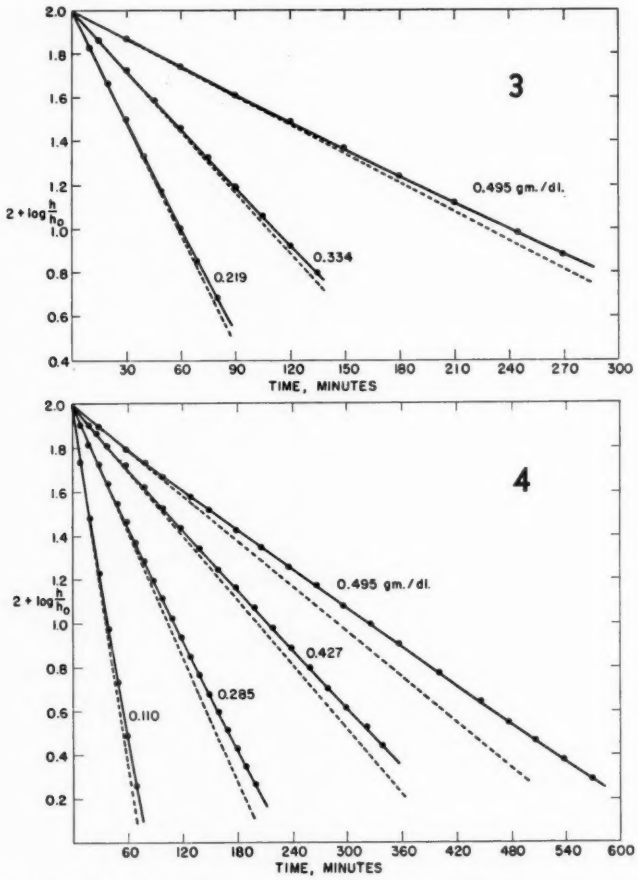


FIG. 3. Plots of log h/h_0 vs. time for three non-Newtonian solutions (natural rubber in benzene) in viscometer MV-2.

FIG. 4. Plots of log h/h_0 vs. time for four non-Newtonian solutions (natural rubber in benzene) in viscometer MV-4.

determining the remaining viscosity–shear points with MV-2, tangents were drawn to the solid lines at various places and their slopes were used to calculate η_{sp}/c values. The mean gradients were calculated from the heads at the points of tangency. The method of arriving at the shear dependence of viscosity using the Chang–Morawetz approach is illustrated by the results presented in Table I for a typical run in the low shear viscometer. Finally, η_{sp}/c values were plotted against D as in Fig. 2.

TABLE I

DETERMINATION OF VISCOSITY–SHEAR RELATION FOR NATURAL RUBBER IN BENZENE AT 25° C. IN MV-2 VISCOMETER*

t, min.	h, cm.	h/h_0	$2+\log h/h_0$	S, sec.$^{-1}\times10^4$†	η_r	η_{sp}/c	D, sec.$^{-1}$
0	4.850	1.000	2.000	—	—	—	—
30	3.600	0.742	1.870	−1.670	19.20	36.8	6.4
60	2.675	0.552	1.742	—	—	—	—
90	2.000	0.412	1.615	−1.637	19.58	37.5	3.5
120	1.490	0.307	1.487	—	—	—	—
150	1.125	0.232	1.365	−1.611	19.90	38.2	1.9
180	0.844	0.174	1.240	—	—	—	—
210	0.640	0.132	1.120	—	—	—	—
245	0.465	0.0959	0.982	—	—	—	—
270	0.375	0.0773	0.888	−1.520	21.10	40.6	0.6
300	0.289	0.0595	0.775	—	—	—	—

*Conc. 0.495 g./dl.
†S_0 (benzene) = -3.204×10^{-3} sec.$^{-1}$.

It should be pointed out that although the curvature in the various log h/h_0 vs. t plots appears slight, the reduced viscosities are nevertheless changing quite rapidly with drop in hydrostatic head. This curvature, of course, becomes progressively less pronounced as the concentration of the rubber solution is diminished. For a concentration of 0.055 g./dl. the plot is so nearly linear that it is quite hazardous to draw tangents to the line, and hence this was not done. However the five-bulb data show that this solution is likewise non-Newtonian. Since the experimental technique is quite simple and inherently capable of a high degree of accuracy, each point in the log h/h_0 vs. t plot must be treated as fully accurate and a smooth line permitted to pass through the various points. The difficulty in this method lies in having to draw tangents to the curves at the various points, and this results in some uncertainty in the particular value calculated for the reduced viscosity at a given gradient. However, there is no uncertainty in the trend of sharply increasing viscosity with reduction in rate of shear, which behavior is in accord with some recent observations of other investigators (5, 7, 8).

Fig. 4 shows typical flow data for several concentrations of natural rubber in benzene obtained in the wider shear range MV-4 viscometer. From these curves η_{sp}/c values at various gradients were calculated in the manner outlined above and plotted in Fig. 2, where they are seen to overlap data obtained with both the MV-2 and five-bulb viscometers. This overlapping strengthens our confidence in the reliability of these results.

An attempt to fit the composite data to the Bueche equation is indicated by the solid lines in Fig. 2. As is clearly seen the agreement is very good. Since this equation was previously shown (3) to represent the reduced viscosity data rather well over the very wide range of 100 to 12,000–20,000 sec.$^{-1}$, the present work has provided therefore experimental confirmation of the correctness of this formula down to extremely small

rates of shear. Moreover, it has furnished a nice technique for evaluating the zero shear reduced viscosity without the necessity of performing an uncertain extrapolation. Essentially, it requires the determination of the parameters of the Bueche formula, which best satisfies the experimental data over a fairly wide range of shear rates. Calculation of the zero shear reduced viscosity from such a derived expression is then immediately possible. An example will illustrate this point. For our purposes, the Bueche equation may be expressed simply as

$$[3] \qquad \frac{\eta_{sp}}{c} = \left(\frac{\eta_{sp}}{c}\right)_0 \left[1 - k\left(\frac{\eta_{sp}}{c} \cdot D\right)^{\frac{1}{2}}\right]$$

where η_{sp}/c and $(\eta_{sp}/c)_0$ are the reduced viscosities at shear rates D and zero, respectively, and k is a constant embracing a number of molecular parameters. Several experimental values of η_{sp}/c and D are substituted into the relation to give an average value of k, and also an approximate value for $(\eta_{sp}/c)_0$. Using these values for k and $(\eta_{sp}/c)_0$, a set of reduced viscosities are then calculated for the different gradients, and compared with the experimental viscosities. These values for k and $(\eta_{sp}/c)_0$ are then varied somewhat and the process repeated until as good a fit as possible of the equation to the data can be found. The value for $(\eta_{sp}/c)_0$ which makes the Bueche equation fit the data best may then be considered as the true zero shear reduced viscosity. Although this point may be open to question, in the absence of any other method for uniquely determining the zero shear reduced viscosity, it is the author's view that the present approach should be adopted as a regular method for obtaining $(\eta_{sp}/c)_0$. This has the obvious advantage of avoiding an unreliable extrapolation in a gradient range where the viscosity is very shear dependent. Typical results obtained by this method are shown in Table II, where

TABLE II

SUITABILITY OF BUECHE EQUATION FOR EXPRESSING SHEAR DEPENDENCE OF VISCOSITY

Data of natural rubber in benzene, conc. 0.427 g./dl., at 25° C.

D, sec.$^{-1}$	η_{sp}/c, exptl.	η_{sp}/c, calc.*
1	31.3	31.2
5	30.0	30.3
10	29.4	29.7
20	28.8	28.8
30	28.3	28.1
40	27.7	27.6
50	27.2	27.0
60	26.7	26.6
70	26.2	26.2

**Calculated assuming $k = 4.2 \times 10^{-3}$ and $(\eta_{sp}/c)_0 = 32.0$.*

the calculated and experimental values for η_{sp}/c are indeed in close agreement. Although this work emphasized the Chang–Morawetz procedure, it is not essential to use it in order to arrive at a reasonable value for $(\eta_{sp}/c)_0$. Actually the data from the five-bulb viscometer are sufficient for this purpose, since in all cases the Bueche plot passes through these experimental points, and hence the zero shear reduced viscosity can be recovered from such viscosity–shear data in the manner outlined above.

ACKNOWLEDGMENTS

The assistance of Mrs. Corinne Bonar with the experimental work is gratefully acknowledged. It is a pleasure to acknowledge also helpful discussions with Dr. R. A. Mock.

REFERENCES

1. BUECHE, F. J. Chem. Phys. **22**, 1570 (1954).
2. CHANG, L. S. and MORAWETZ, H. J. Polymer Sci. **13**, 414 (1954).
3. GOLUB, M. A. J. Phys. Chem. **60**, 431 (1956).
4. GOLUB, M. A. J. Phys. Chem. (In press).
5. KRIGBAUM, W. R. and FLORY, P. J. J. Polymer Sci. **11**, 37 (1953).
6. MOCK, R. A. and MARSHALL, C. A. J. Polymer Sci. **14**, 217 (1954).
7. PETERLIN, A. and SIGNER, R. Helv. Chim. Acta, **36**, 1575 (1953).
8. SIGNER, R. and BERNEIS, K. Makromol. Chem. **8**, 268 (1952).

STRUCTURE OF A HEMICELLULOSE FROM MAPLE WOOD PREVIOUSLY EXTRACTED BY LIQUID AMMONIA[1]

L. G. NEUBAUER[2] AND C. B. PURVES

ABSTRACT

The extraction of maple wood meal with anhydrous liquid ammonia under pressure near 20° altered the wood in such a way that an additional small amount of lignin could be extracted with ethanol, and 1.9% of crude hemicelluloses with hot water. An additional 0.25% of nitrogen was retained, apparently as insoluble amides, by the residual wood. After elimination of pectic material by acetylation, the three subfractions of the hemicellulose acetate had identical specific rotations of $-61\pm1°$ in chloroform; all three corresponded in composition to a combination of one methylglucuronic anhydride to six anhydroxylose units. A conventional study by the methylation method suggested that the hemicellulose was a branched-chain structure averaging four anhydroxylose units linked 1—4, with one also substituted in the second position, and another linked 1—3.

INTRODUCTION

The observation (32) that nearly anhydrous (99.5%) liquid ammonia under pressure at room temperature extracted most of the acetyl groups in maple wood flour as acetamide (4) suggested that any other type of ester group in the wood might also have been cleaved to an acid amide. If such were the case, then components of the wood that had not been removed in the exhaustive preliminary extractions with alcohol, ether, and

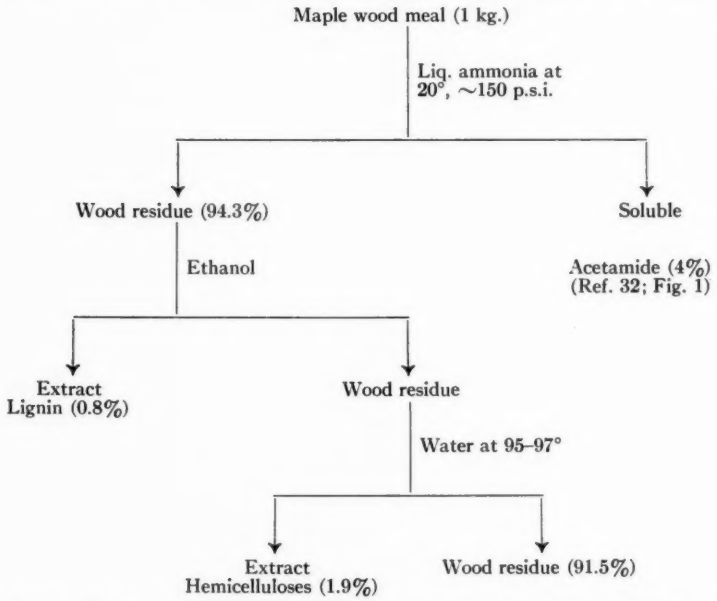


FIG. 1. Extraction of maple wood meal with liquid ammonia, ethanol, and water in succession. All percentages based on original, solvent-extracted wood meal.

[1]*Manuscript received December 21, 1956.*
Contribution from the Division of Industrial and Cellulose Chemistry, McGill University, and the Wood Chemistry Division, Pulp and Paper Research Institute of Canada, Montreal, Que. Abstracted from a Ph.D. thesis submitted to the University by L. G. N. in September, 1949.
[2]*Present address: Tasman Pulp and Paper Company, Bay of Plenty, New Zealand.*

water might have acquired solubility in one or other of these liquids. The present research originated in attempts to explore this possibility.

Two samples of the same wood meal were analyzed in an air-dry condition before and after the extraction with liquid ammonia (Fig. 1), the actual work being performed by Yan and by one of the writers in different years. As Table I shows, the residual wood

TABLE I

COMPOSITION* OF MAPLE WOOD MEALS BEFORE AND AFTER EXTRACTIONS

Sample	Klason lignin, %	Acetyl, %	N,† %	Yield, %
First wood meal				
Before liq. NH_3‡	23.5	4.8	0.08	100
After liq. NH_3§	21.8	1.3	0.43	94.3
Second wood meal				
Before liq. NH_3	23.5	3.9	0.09	100
After liq. NH_3	21.1	0.43	0.33‖	94.3
After EtOH and H_2O extractions	20.2	0.42	0.33‖	91.5

**All percentages based on original solvent-extracted wood meal and corrected for moisture content of samples. First sample analyzed by Yan.*

†Semimicro-Kjeldahl procedure.

‡Ash, 0.36, 0.35%.

§Ash, 0.35, 0.34%.

‖N, 0.23% as NH_3*, expelled by hot aqueous* NaOH; N, *0.01% as* NH_3*, expelled by aqueous* MgOH.

meals were slightly deficient in Klason lignin, markedly deficient in acetyl groups, and had acquired additional nitrogen. This additional nitrogen was probably not attributable to retained acetamide, because exhaustive extractions of the second wood sample with alcohol and then with hot water failed to remove it, although they did remove 0.8% of a lignin and 1.9% of a crude hemicellulose. When samples were distilled with dilute aqueous sodium hydroxide, however, the surplus nitrogen was removed as ammonia, and therefore was present in the wood residue as an amide or an ammonium salt. The latter possibility was improbable because boiling aqueous magnesium hydroxide expelled no more ammonia from the residue than did boiling water (22). The simplest interpretation to put upon the data was that some of the uronic acid groups in the wood were transesterified with other constituents; cleavage of these ester groups by liquid ammonia made it possible to extract small additional quantities of lignin and carbohydrate material by the renewed use of alcohol and water, while the insoluble amides remained in the residue. Rogers, Mitchell, and Ritter (27) found 4.36% of uronic acid, or 0.25 milliequivalents per gram, in a maple wood which contained only 0.24% of ash. Even the unlikely assumption that all this ash was sodium carbonate failed to raise the calculated ash alkalinity above 0.045 milliequivalent per gram. Since wood is nearly neutral in reaction, much of the uronic acid must have been neutralized in a way not dependent on salt formation. The transesterification hypothesis would explain this anomaly also. Other reasons led Anderson and co-workers (1) to conclude that pectic substances in woods might be partly esterified with other plant constituents, while Foster, Schwerin, and Cohen (11) formed the same opinion about the uronic anhydride units in *Eucalyptus regnans*.

Concentration of the combined aqueous extract (Fig. 1), followed by dilution with ethanol, caused the precipitation in 1.9% yield of a gray powder which consisted essentially of uronic anhydride and pentosan units. A long series of fractional precipitations from aqueous alcohol tended to concentrate the pentosan in the more soluble portion, but analytical constancy in successive subfractions was never attained. Selective absorption on carbon was equally unsatisfactory. Finally, subfractions were combined in the three groups, *M*, *X*, and *Y*, whose divergent analyses were recorded in Table II.

TABLE II

ANALYSES* OF HEMICELLULOSE FRACTIONS AND THEIR ACETATES

Fraction	Yield,† %	Uronic anhydride, %	Anhydro-xylose,‡ %	Methoxyl, %	Per cent pectic acid§	Periodate reduced, M.‖
M	27	25.8	65.9	2.5	10	3.9
X	13	43.3	50.4	1.9	32	4.1
Y	7	23.3	74.3	2.9	7	4.0
Acetates¶					Acetyl	$[\alpha]_D^{20}$
M	68**	11.3	51.7	2.0	36.4	−59.8°
X	77	11.4	51.1	2.0	36.5	−60.6°
Y	118	11.4	51.7	2.0	36.5	−61.9°

**Averages of concordant duplicate determinations. Corrected for ash of 3.3% or less, except in M, where the ash was 15.8%.*

†By weight.

‡Corrected for 21.5% yield of furfural by weight from uronic acid content.

§Estimated from the excess of the observed uronic anhydride over that required for a hemicellulose based on six anhydroxylose to each methylglucuronic anhydride unit. Calc. for $6C_5H_8O_4.C_7H_{10}O_6$ *(mol. wt. 982): uronic anhydride, 17.9; anhydroxylose, 80.7;* OCH_3*, 3.2%.*

‖Per mole of hemicellulose of ml. wt. 982. Corrected for reduction by pectic acid and for production of formic acid.

¶Ash, 0.3% or less; specific rotations in chloroform, c, 0.9–1.1% Calc. for $6C_5H_8O_4.C_7H_{10}O_6.13CH_2CO$ *(mol. wt. 1528): uronic anhydride, 11.5; anhydroxylose, 51.8; methoxyl, 2.0; acetyl, 36.6%.*

***Also 36% of acetate insoluble in acetone and in water.*

Each of these groups was then acetylated with pyridine as catalyst, and dilution of the acetylation mixture precipitated acetates of the same composition and optical rotation but in differing yield. The composition of the unprecipitated portion approximated that of a polyhexuronic acid diacetate, and the isolation of mucic acid by the hydrolysis and oxidation of the original polysaccharide showed that the polyhexuronic acid was pectic acid. Hirst and Jones (15) discovered that pectic acid could be separated in the same way, or by methylation, from plant gums and crude pectins. Anderson and his collaborators (1) isolated up to 3% of pectic material from various woods, and showed that it was a contaminant in an aspen hemicellulose extracted by alkali (2).

The analyses of the uniform acetate were those required by the acetate of a hemicellulose averaging six pentosan units to one of methoxyhexuronic anhydride. If it was assumed that the fractions *M*, *X*, and *Y* consisted only of this hemicellulose and of pectic acid, then the percentage amount of the latter could be calculated from their uronic acid contents (Table II, column 6). These percentages in turn made it possible to correct the observed consumption of periodic acid by the fractions *M*, *X*, and *Y* for the amount consumed by their content of pectic acid. The results (right hand column) indicated that the repeating unit of the hemicellulose reduced 4 moles of periodic acid rapidly with the production of no formaldehyde and probably of no formic acid. A sample

of fraction *M* was hydrolyzed with boiling 3% sulphuric acid until the copper reducing power attained a maximum value equivalent to the liberation of 63.7% of xylose. The amount of crystalline xylose isolated from the hydrolyzate and carefully identified was 56.3%, and no other sugar could be detected. These observations strongly suggested that all of the pentosan in fraction *M* was xylan, and that the hydrolysis yielded 4 moles of xylose and 1 mole of an aldotriuronic acid which was not isolated.

When fraction *M* was thoroughly methylated by standard methods, the product was readily separated into a chloroform-soluble portion with the composition required by the above hemicellulose when completely substituted, and a chloroform-insoluble portion which appeared to be a methylated salt of pectic acid. Oxidation of the fully methylated polysaccharide with nitric acid yielded a pale yellow sirup which was esterified with methanolic hydrogen chloride and then fractionally distilled. The methyl ester of 2,3,4-tri-*O*-methyl saccharolactone crystallized spontaneously from the distillate. When the methanolic mother liquors were saturated with ammonia gas the diamides of the optically active 2,3-di-*O*-methylxyloglutaric and of the internally compensated 3-*O*-methylxyloglutaric acid were recovered in a crystalline condition and suitably identified. The final mother liquors yielded an uncrystallized substance which gave a negative Weerman (30) test for α-hydroxy acids, and which had the composition of a di-*O*-methylxyloglutaramide. This substance appeared to be the internally compensated 2,4-isomer. Although the distillation of the original mixture of methyl esters was not quantitative, the percentage amounts of the above four compounds actually isolated were in close agreement with those required by a molar ratio of 1: 4: 1: 1.

Both anhydroxylose and anhydroglucuronic acid appear to occur in natural polysaccharides invariably with pyran cyclic structures. If X and G, respectively, represented these structures, then the results of the methylation and hydrolysis just described were compatible with the branched-chain formula

$$\begin{array}{l}\text{—4X1—4X1—4X1—4X1—4X1—}\\ \qquad\qquad\quad\ \ \mathbf{2}\\ \qquad\qquad\quad\ \ \text{1X3—1G}\end{array}$$

This formula was not unique because the anhydroxylose units could be interchanged, and the branch lengthened or shortened, in various ways consistent with the experimental data. The four anhydroxylose units linked 1—4 each retained unsubstituted hydroxyl groups in the second and third positions, and would account for the observed consumption of 4 moles of periodate by the structure shown in the formula. This inference suggested that the anhydroglucuronic acid G did not react with periodate and therefore did not contain adjacent hydroxyl groups in the second, third, and fourth positions. The assumption that the methyl group in the hemicellulose was present in the third position of the anhydroglucuronic unit satisfied this requirement (9) and was also consistent with the repeated failure to find unmethylated saccharolactone among the oxidation products of the hemicellulose.

Previous investigations on the hemicelluloses from maple wood appear to be restricted to the preliminary fractionations and group analyses of Haas (12) and of Ritter and his colleagues (21, 27). The ratio of six anhydroxylose to one methylglucuronic acid residue now found has been encountered among the more soluble fractions of hemicelluloses derived from oak (24), jute (28), and other plants. To judge from a recent review (14), a branched chain hemicellulose is not unusual, and glucuronic acid residues are usually

present either as such or as the 4-*O*-methyl derivative. Since 3-*O*-methylglucuronic acid residues have been thought to be present only in the isolated case of jute hemicellulose (7, 8), the presence of this isomer in the product from maple requires to be confirmed by a more direct method than the one used in the present research.

EXPERIMENTAL

Materials

The wood meal, of 40 to 80 mesh, was prepared from the log of sugar maple (*Acer saccharum* Marsh) used in the previous research (32) and was exhaustively extracted with distilled water, ethyl ether, and ethanol in succession. Little or no "native lignin" was removed by the last two liquids. After being dried in the air, the meal was extracted for 5 hours at room temperature with five times its weight of nearly anhydrous liquid ammonia in the apparatus constructed for that purpose (32). Acetamide was separated in 85% of the theoretical yield from the extract, together with a lignin and a polyuronide in yields amounting to 1.1% and 1.2%, respectively, of the wood.

The air-dry, ammonia-extracted wood meal, 2.25 kg., was thoroughly extracted in a Soxhlet apparatus with ethanol, and after being dried once more in the air, each 500 g. was extracted three times with 3 liter volumes of distilled water at 95–97° for 2 hours. Residual wood meal, 2.25 kg., saved from the earlier work was treated in the same fashion. After being evaporated, the combined ethanol extracts from the 4.5 kg. of wood yielded 41 g. of a dark brown, resinous material which gave a positive test for angiosperm lignin in the Mäule reaction. The aqueous extracts from each 500 g. batch were centrifuged to remove turbidity, were combined, and were evaporated *in vacuo* to 750 ml. Two volumes of ethanol were then added, and the resulting precipitate was recovered by filtration, solvent-exchanged through ethanol and ether, and dried *in vacuo* at 50° over phosphoric anhydride. Total yield of crude hemicelluloses, 91.5 g. or 1.9% of the wood. A pale brown solid, 11.4 g., was recovered from the mother liquors.

The analyses of the residual wood meal at various stages of extraction were summarized in Table I. Acetyl groups were determined after steam distillation of a solution of the sample in 72% sulphuric acid (19); total nitrogen by the Gunning (3) modification of the semimicro-Kjeldahl method; amide nitrogen by distillation of 0.5 g. samples with 20 cc. of 0.001 *N* sodium hydroxide; and ammonium salt nitrogen by distillation with water containing 0.3 g. of magnesium oxide (22). In the last three cases the ammonia was distilled into a suitable amount of standard hydrochloric acid, and the whole was back-titrated to a methyl red end point with 0.01 *N* alkali.

Examination of the Hemicellulose (Fig. 1 and Table II)

The pale gray powder, 91.5 g., was precipitated several times from aqueous solution by two volumes of ethanol, but the composition of the product showed no signs of becoming constant and its solution in water was still too turbid to permit an optical rotation to be determined. Absorbent carbon, 25 g., was added to a solution of the less soluble portion of the product, 75 g., in 3 liters of hot water, but only 28.9 g. of a very pale gray powder was recovered from the filtrate. After being fractionated from aqueous ethanol, the less soluble portion was six times reprecipitated in the same way, and yielded 11.6 g. of the less soluble subfraction *X*. The more soluble portion, *Y*, 6.6 g., was a white powder. Since the portion of the hemicellulose that remained adsorbed on the carbon

could not be eluted with water, this operation was carried out, although with difficulty, by extraction with cold 1% sodium hydroxide. The eluate was neutralized with dilute hydrochloric acid, and then diluted with two volumes of ethanol to precipitate 28.1 g. of a pale gray powder. The product was combined with several minor fractions and twice reprecipitated to yield 24.8 g. of fraction *M*. McCready, Swenson, and Maclay's semimicro method (20) was used to determine uronic anhydride, and that of Klein and Acree (18) for furfural. The percentage of the latter was reduced by 21.5% of the percentage of uronic acid found (23) and the result when multiplied by the factor 1.557 was accepted as per cent anhydroxylose units.

Samples, 1 g.–1.3 g., of the three fractions *X*, *Y*, and *M* were separately acetylated by adding 20 cc. of acetic anhydride to a suspension previously swollen in pyridine. The mixtures were kept at room temperature for 24 hours, then near 100° for 4 hours, and were poured into 400 ml. volumes of ice and water. The precipitates were extracted with acetone, any small residue being removed by filtration, and after being concentrated the extracts were diluted with water. When dried *in vacuo* at 60° over phosphorus pentoxide, the hemicellulose acetates were hard, white powders. Clark's semimicro method (6) was used to determine acetyl content, and the values given in Table II were not increased by reacetylating the acetates. The aqueous residues from the above acetylations on evaporation yielded three gray powders containing 10–47% of ash which was apparently silica. When corrected for this ash, the yields were 35%, 9%, and 16% from fractions *X*, *Y*, and *M*, respectively; their uronic anhydride content was 62.5–69.7%; anhydroxylose, 2.0–3.2%; and acetyl, 24.9–31.5%. Calc. for pectic acid diacetate: uronic anhydride, 67.7; acetyl, 33.1%.

A 0.04 *N* solution of sodium metaperiodate was used to oxidize 0.3 g. samples of the three unacetylated hemicellulose fractions, the consumption of oxidant being followed by the arsenite (10) method, and the production of formic acid by titration to the original pH of the solution with standard alkali. None of the fractions yielded enough formaldehyde to be detected by the dimedon reagent (26). By extrapolating the later, nearly linear portions of the plots to zero time, the rapid initial reactions were found to consume 5.5, 4.7, and 4.53 moles of periodate per 1000 g. of fractions *X*, *Y*, and *M*, respectively, and to produce 0.43, 0.27, and 0.23 moles of formic acid. In the case of 1000 g. of fraction *M*, the 101 g. of pectic acid this amount was assumed to contain (Table II) would reduce 1 mole of periodate per galacturonic anhydride unit (base mol. wt. 176) or 0.57 mole. By attributing the production of 0.23 moles of formic acid to a rapid secondary oxidation of the pectic material, the latter consumed a further 0.46 mole of periodate. The consumption attributable to 899 g. of hemicellulose was therefore $(4.53-0.57-0.46)$ 3.5 moles, or 3.9 moles for the base molecular weight of 982 g. assumed for the hemicellulose. The corresponding values given in Table II for fractions *X* and *Y* were estimated in the same way.

A portion of fraction *M*, 3.38 g. (ash-free), was hydrolyzed near 97° in 250 cc. of 3% sulphuric acid until the Shaffer–Hartmann–Somogyi (29) copper reducing power reached a maximum corresponding to 2.16 g. of xylose (63.5%) after 160 minutes. After filtration from 0.11 g. of a dark material, a 5 cc. aliquot of the filtrate was neutralized with sodium hydroxide and was found to be unfermented by yeast; added glucose was later fermented readily. Glucose, fructose, and mannose were therefore absent. The remainder of the hydrolyzate was neutralized with barium carbonate, and the filtrate, now free of sulphate

ion, was concentrated and diluted with 10 volumes of ethanol. A light brown precipitate (0.12 g.) which formed was isolated and oxidized with nitric acid according to Wise and Peterson (31). The yield of 0.035 g. of insoluble white crystals melted at 217°, undepressed by admixture with authentic mucic acid of melting point 217°. When the filtrate from the above brown precipitate was evaporated *in vacuo*, 2.81 g. of a pale yellow, dextrorotatory, partly crystalline solid remained. A 0.37 g. portion when analyzed for xylose by the method of Breddy and Jones (5) yielded 0.32 g. of the dimethyl acetal of dibenzylidene-D-xylose with the correct specific rotation of $[\alpha]_D^{20}$ −8.5° in chloroform and melting at 210°. A mixed melting point with an authentic sample was at 211°. This quantity of acetal was equivalent to 56.3% by weight of xylose. Tests on 0.3 g. samples for arabinose, rhamnose, and galactose by the method of Hirst, Jones, and Woods (16) were completely negative.

Heidelberger and Goebel's method (13) was used to oxidize 1.17 g. (ash-free) of fraction *X* with 50 cc. of boiling *N* hydrobromic acid containing 0.5 cc. of bromine. After being kept 24 hours under reflux, the liquor was evaporated, finally over solid potassium hydroxide, to 3 ml., and 0.315 g. of mucic acid was removed and identified. The mother liquor was neutralized with potassium hydroxide and concentrated to a sirup. No crystals of acid potassium saccharate could be obtained when this sirup was diluted with glacial acetic acid, although as little as 40 mg. of the salt could be readily isolated by the procedure used.

Methylation and Hydrolysis of Fraction M

Following the procedure of Hirst and Jones (15), 3.31 g. (ash-free) of the hemicellulose *M* was mixed with 30 cc. of 4 *N* thallous hydroxide; the precipitate which immediately formed was separated and dried at 35° *in vacuo*, was crushed to a pale yellow powder, 10.3 g., and was warmed under reflux with 25 cc. of methyl iodide for 18 hours. The methyl iodide was then evaporated, and the residue remethylated in the same way. The third methylation involved the use of 20 cc. of anhydrous ethanol and 50 cc. of *N* thallous ethylate in benzene (25), and the fourth, with 50 ml. of the thallous ethylate solution, failed to increase the methoxyl content of the product. Exhaustive extraction of this product with chloroform removed 2.75 g. of a very pale yellow resin. Found: uronic anhydride 15.0, 15.0; methoxyl, 39.3, 39.4; ash, 0.1%. Calc. for the methyl ester of the fully methylated hemicellulose, $6C_6H_8O_4.C_6H_8O_6.15CH_2$ (mol. wt. 1178): uronic anhydride, 14.9; methoxyl, 39.5%. The residue, insoluble in chloroform, on re-extraction with hot water, yielded 0.38 g. of a gray powder. Found: uronic anhydride, 51.4, 51.5; methoxyl, 28.6, 28.7; ash, 20.1, 20.2%.

Simultaneous oxidation and hydrolysis of the chloroform-soluble product, 2.29 g., was by solution at 80° for 6 hours in 35 cc. of 32% nitric acid (17). The clear, pale orange solution was evaporated with the simultaneous addition of water until the acidity was reduced to pH 6, and was then concentrated to a sirup. After being esterified by 8 hours' boiling with 35 cc. of 3% methanolic hydrogen chloride, the ether-soluble, neutral, almost colorless esters, 1.74 g., were distilled at 0.1 mm. pressure; fractions 1, 2, and 3 weighed 0.22, 0.69, and 0.39 g., and boiled at 110–129°, 130 145°, and 146–160°, respectively. The still residue, 0.31 g., was dark brown.

Fraction 3 partly crystallized on standing, and fraction 2 when triturated with ethanol–ether–ligroin (1:1:2 vol.). After one recrystallization from ether, both crops melted at 106–107° and both melting points were undepressed by admixture with the authentic

methyl ester of 2,3,4-tri-*O*-methylsaccharolactone (prepared from β-glucosan ⟨1,4⟩ ⟨1,5⟩), m.p. 109°. Total yield from 1.3 g. of distillate, 0.15 g. or 11.5%. The mother liquors from the two crops were separately evaporated to dryness and the residues redissolved in 5 to 8 cc. of anhydrous methanol saturated with dry ammonia gas. The two crystalline crops that separated after 2 days at 0°, 0.06 and 0.07 g., were recrystallized from aqueous methanol and both melted at 161–162° (dec.), a mixed melting point being undepressed. These crystals had the methoxyl and nitrogen contents required by a mono-*O*-methyl xyloglutardiamide, were optically inactive, and gave a positive Weerman (30) test for α-hydroxy acid amides. They consisted of 0.13 g. (10%) of 3-*O*-methyl xyloglutardiamide. The original mother liquors from the two crops were each evaporated to one-half volume and left for 3 days at 0°. Each deposited identical crystals melting at 140° after recrystallization from aqueous methanol; yields 0.10 g. and 0.39 g., respectively. These crystals gave a positive Weerman test, had a specific rotation $[\alpha]_D^{20}$ +28° in water (*c*, 0.8), and had the methoxyl and nitrogen contents of a di-*O*-methyl xyloglutardiamide. They were therefore the 2,3-di-*O*-methyl isomer.

A solution of fraction 1 in 3 cc. of methanol saturated with ammonia gas deposited 0.04 g. of dimethoxysuccinamide melting correctly at 290° (dec.) after one recrystallization from water, and with the correct methoxyl content. After a further 6 days at 0°, the methanolic mother liquor deposited a further 0.14 g. of 2,3-di-*O*-methyl xyloglutardiamide (m.p. and mixed m.p. 139–140°), the total yield of which was therefore 0.63 g. or 48.5%. No further crystallization occurred in the final mother liquors from the three fractions of the original distillation. When combined and evaporated, they yielded 0.14 g. (11%) of a brown sirup which gave a negative Weerman test. Found: OCH_3, 29.0; N, 13.8%. Calc. for $C_7H_{14}O_5N_2$: OCH_3, 30.1; N, 13.6%. The sirup was probably 2,4-di-*O*-methyl xyloglutardiamide.

ACKNOWLEDGMENT

One of us (L.G.N.) wishes to thank the Council for Scientific and Industrial Research, New Zealand, for financial help during the research.

REFERENCES

1. Anderson, E., Seigle, L. W., Krznarich, P. W., Richards, L., and Marteny, W. W. J. Biol. Chem. **121**, 165 (1937).
2. Anderson, E. and Wise, L. E. Paper Ind. and Paper World, **27**, 1037 (1945).
3. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 6th ed. Washington, D.C. 1947. p. 28.
4. Bishop, C. T. Can. J. Chem. **30**, 229 (1952).
5. Breddy, L. J. and Jones, J. K. N. J. Chem. Soc. 738 (1945).
6. Clark, E. P. Ind. Eng. Chem. Anal. Ed. **9**, 539 (1937).
7. Das Gupta, P. C. and Sarkar, P. B. Textile Research J. **24**, 705 (1954).
8. Das Gupta, P. C. and Sarkar, P. B. Textile Research J. **24**, 1071 (1954).
9. Douglas, I. B. Paper Trade J. **131**, 99 (Nov. 30, 1950).
10. Fleury, P. and Lange, J. J. pharm. chim. **17**, 196 (1933).
11. Foster, D. H., Schwerin, G., and Cohen, W. E. Australian J. Sci. Research, **3A**, 504 (1950).
12. Haas, H. Makromol. Chem. **2**, 217 (1948); Chem. Abstracts, **43**, 5587 (1949).
13. Heidelberger, M. and Goebel, W. F. J. Biol. Chem. **74**, 613 (1927).
14. Hirst, E. L. J. Chem. Soc. 2974 (1955).
15. Hirst, E. L. and Jones, J. K. N. J. Chem. Soc. 496 (1938).
16. Hirst, E. L., Jones, J. K. N., and Woods, E. A. J. Chem. Soc. 1048 (1947).
17. Hirst, E. L. and Purves, C. B. J. Chem. Soc. 1352 (1923).
18. Klein, G. M. and Acree, S. F. Bur. Standards J. Research, **8**, 25 (1932).
19. Lemieux, R. U. and Purves, C. B. Can. J. Research, B, **25**, 485 (1947).
20. McCready, R. M., Swenson, H. A., and Maclay, W. D. Ind. Eng. Chem. Anal. Ed. **18**, 290 (1946).
21. Mitchell, R. L., Rogers, S. C., and Ritter, G. J. Ind. Eng. Chem. **40**, 1528 (1948).

22. MULLIKEN, S. P. A method for the identification of pure organic compounds. Vol. 2. John Wiley and Sons, Inc., New York. 1916. p. 22.
23. NORRIS, F. W. and RESCH, C. E. Biochem. J. **29**, 1590 (1935).
24. O'DWYER, M. H. Biochem. J. **28**, 2116 (1934).
25. PURVES, C. B. and HUDSON, C. S. J. Am. Chem. Soc. **59**, 49 (1937).
26. REEVES, R. E. J. Am. Chem. Soc. **63**, 1476 (1941).
27. ROGERS, S. C., MITCHELL, R. L., and RITTER, G. J. Ind. Eng. Chem. Anal. Ed. **19**, 1029 (1947).
28. SARKAR, P. B., MAZUMDAR, A. K., and PAL, K. B. Textile Research J. **22**, 529 (1952).
29. SOMOGYI, M. J. Biol. Chem. **70**, 599 (1926).
30. WEERMAN, R. A. Rec. trav. chim. **37**, 16 (1918).
31. WISE, L. E. and PETERSON, F. C. Ind. Eng. Chem. **22**, 362 (1930).
32. YAN, M. M. and PURVES, C. B. Can. J. Chem. **34**, 1747 (1956).

SOME OXIDATION REACTIONS OF DELCOSINE[1]

RAGINI ANET,[2] D. W. CLAYTON,[2] AND LÉO MARION

ABSTRACT

The alkaloid delcosine was oxidized by chromic acid in acetic acid, and also by the Oppenauer reaction, to dehydrodelcosine in which the carbonyl was shown by infrared absorption to be in a pentatomic ring. Oxidation of the alkaloid with silver oxide gave two products: (*a*) N-desethyldelcosine, which could be N-acetylated or converted back to the original base by ethylation, thus proving the presence of an N-ethyl group; (*b*) a compound, $C_{24}H_{37}O_7N$, the properties of which agreed best with those of an internal ether, i.e., anhydrohydroxydelcosine. The action of N-bromosuccinimide on the alkaloid produced the same two compounds as silver oxide, plus a derivative, $C_{22}H_{33}O_7N$, that proved to be N-desethyl-anhydrohydroxydelcosine. Potassium permanganate oxidized delcosine to a product, $C_{22}H_{31}O_7N$, that had lost the N-ethyl group, contained the internal ether, and also a carbonyl in a five-membered ring. This same product was obtained on similar oxidation of N-desethyl-anhydrohydroxydelcosine. Oxidation of delcosine with mercuric acetate gave N-desethyldelcosine and N-desethyl-anhydrohydroxydelcosine, together with a compound that was very soluble in water and proved to be the carbinolamine formed by hydroxylation of the methylene in the N-ethyl group. These results are discussed in terms of a structure that is tentatively advanced for delcosine.

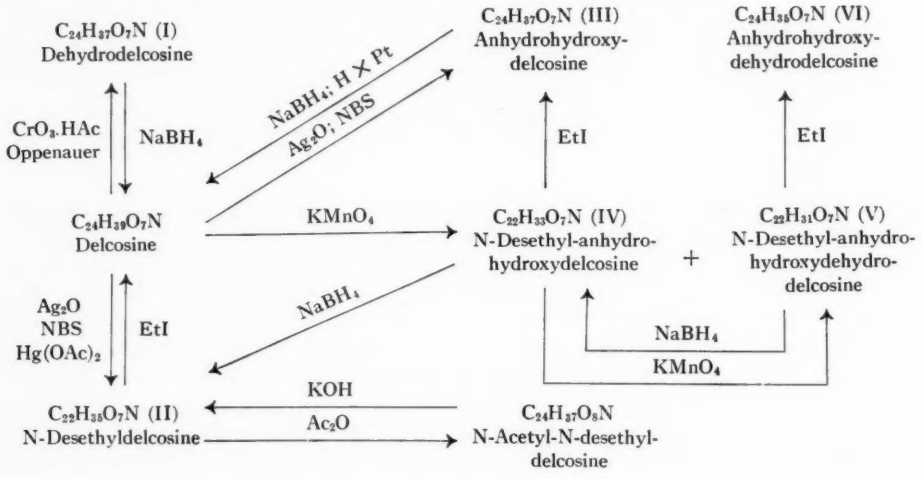
Delcosine is a monoacidic base with an empirical formula that has been previously established as $C_{24}H_{39}O_7N$ (12). Of its seven oxygens three are known to be present in methoxyl groups and four in hydroxyls. Two of the hydroxyls are readily acetylated, but the remaining two are not (12), and are probably tertiary alcoholic groups. It does not take up any hydrogen in the presence of Adams' catalyst, thus indicating the absence of unsaturation. The ultraviolet spectrum of the bases, like that of lycoctonine (4), shows only end absorption and is similar to that of sparteine, which contains no unsaturation. As with lycoctonine, the end absorption is due to the basic nitrogen, with some contribution from hydroxyls and methoxyls (4). The infrared spectrum of delcosine does not contain any absorption band characteristic of a double bond. Delcosine must, therefore, possess a structure made up of six rings and, further, it has been shown by Goodson (7) probably to contain an N-ethyl group.

The behavior of delcosine towards various oxidizing agents has been investigated, and the results together with their interpretation in terms of a tentative structure are now reported.

Oxidation of delcosine with chromic acid in acetic acid or in dilute sulphuric acid gave a ketone $C_{24}H_{37}O_7N$ (I) in poor yield. The ketone formed mixed crystals with delcosine, but could be separated by paper chromatography or by countercurrent distribution. Its infrared spectrum (band at 1755 cm.$^{-1}$, no C—H stretching absorption at 2600–2800 cm.$^{-1}$) revealed it to be a five-membered ketone. It formed a monoöxime showing no carbonyl absorption in the infrared. Hence one of the hydroxyls in delcosine must be secondary and present in a five-membered ring. Attempts to condense the ketone I (dehydrodelcosine) with piperonal failed and, hence, it is probable that no methylene groups are present adjacent to the carbonyl. The same dehydrodelcosine was obtained by Oppenauer oxidation but the yield was also low and attempts to improve it by varying the conditions (cf. 13) proved fruitless.

[1]*Manuscript received December 17, 1956.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 4265.
[2]*National Research Council of Canada Postdoctorate Fellow.*

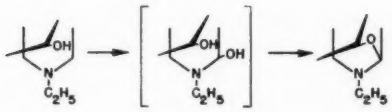
Delcosine was found to be inert to silver oxide under conditions which gave hydroxylycoctonine from lycoctonine (4). If, however, the reaction was carried out at 80° in ethanol for 68 hours, two products were obtained. One of these, $C_{22}H_{35}O_7N$ (II), was sparingly soluble in benzene; it contained five active hydrogens and was a stronger base (pK_a 7.9) than delcosine (pK_a 6.49). With acetic anhydride and pyridine it formed a neutral acetyl derivative showing an amide carbonyl absorption peak at 1643 cm.$^{-1}$ in the infrared. The acetyl derivative on hydrolysis gave back the basic product II, which must therefore be a secondary base. The base II was shown to be N-desethyldelcosine by treatment with ethyl iodide in dimethylformamide, which converted it to delcosine, thus firmly establishing the presence of an N-ethyl group in the alkaloid.



The second product of the silver oxide oxidation, $C_{24}H_{37}O_7N$ (III), was a much weaker base than the first (pK_a 4.2). Its infrared absorption spectrum showed no carbonyl nor double bond absorption, but contained sharp peaks at 1000 and 900 cm.$^{-1}$ which were absent in the spectrum of delcosine. As the pK_a of III showed a sharp drop of 2.29 units from that of delcosine, a change in the environment of the nitrogen such as the formation of a carbinolamine was suspected. (Lycoctonine on conversion to hydroxylycoctonine shows a drop in pK_a of three units.) The analysis, however, which showed that III contained the same number of oxygens as delcosine, and the fact that the compound formed a perchlorate that was not an anhydronium salt, provided evidence that compound III was not a simple carbinolamine.

If, on the other hand, it were assumed that one of the hydroxyls in the molecules was placed suitably to react with the carbinolamine formed initially, there could then arise an internal ether as indicated in the following partial formulae, and this would be in agreement with the pK_a of the compound and its analytical figures. In fact, compound III contained only three active hydrogens, thus substantiating the assumption. Cyclic carbinolamine internal ethers are not unknown in the field of alkaloids (cf. 11).

Reduction of compound III with sodium borohydride gave back delcosine, thus showing that no change had taken place in the stereochemistry of the molecule. Although



compound III was inert to hydrogenation in neutral medium, it rapidly took up 1 mole of hydrogen in aqueous perchloric acid in the presence of Adams' catalyst to give delcosine. There is thus no doubt that the net result of the oxidation was the abstraction of two hydrogens. Hence the reaction can be rationalized only on the basis of an internal ether formed from an intermediate carbinolamine.[3] Thus compound III is anhydrohydroxydelcosine (see partial formulae above).

N-Bromosuccinimide is known to oxidize secondary alcohols to ketones (6), and has been used in such a reaction by Cookson and Trevett (2) in their study of delpheline. Delcosine when oxidized with this reagent in buffered solution gave three products. One was identical with anhydrohydroxydelcosine (III) described above. The second product, $C_{22}H_{33}O_7N$ (IV), had a pK_a 4.6 and contained four active hydrogens. On treatment with ethyl iodide in dimethyl formamide it gave rise to III. Hence compound IV was clearly the internal ether of N-desethyldelcosine, i.e., N-desethyl-anhydrohydroxydelcosine. Reduction of IV with sodium borohydride gave N-desethyldelcosine (II), which was also obtained as the third product of the oxidation. The oxidation with N-bromosuccinimide was thus not specific, but attacked two carbon atoms vicinal to the nitrogen.

Oxidation of delcosine with potassium permanganate in acetone gave intractable material and some neutral product which could not be purified. On the other hand, potassium permanganate in N-potassium hydroxide at room temperature oxidized delcosine to two products. One was N-desethyl-anhydrohydroxydelcosine IV also obtained in the oxidation of delcosine with N-bromosuccinimide. The other was a high melting compound, $C_{22}H_{31}O_7N$ (V), the infrared absorption spectrum of which in nujol contained a band at 1741 cm.$^{-1}$ which shifted in chloroform solution to 1751 cm.$^{-1}$ indicating the presence of a five-membered cyclic ketone. In addition, the spectrum in nujol contained bands at 1000 and 900 cm.$^{-1}$ which were present in the spectra of III and IV, but absent in the spectra of delcosine, dehydrodelcosine (I), and N-desethyldelcosine, and can be considered as characteristic of the internal ether. Brief treatment with sodium borohydride reduced compound V quantitatively to N-desethyl-anhydrohydroxydelcosine IV. Hence the compound V was N-desethyl-anhydrohydroxy-dehydrodelcosine.[4]

N-Desethyl-anhydrohydroxydelcosine IV could be oxidized in over 60% yield to the corresponding ketone V. This increased yield indicated that in the oxidation of delcosine to the ketonic compound V, the formation of IV is the controlling factor. This oxidation is significant since secondary alicyclic alcohols do not give ketones in good yield under these conditions. The relatively high yield obtained may indicate that the secondary hydroxyl involved in the reaction is flanked on either side by quaternary carbons or, alternatively, by carbons located at bridgeheads, so that the ketone once formed cannot enolize.

The N-desethyl ketone V when ethylated with ethyl iodide gave rise to a new ketone,

[3]*It is possible to explain the analysis and the active hydrogen on the basis of hemiketal structure but the basic strength of compound III is incompatible with this. Furthermore, the infrared spectrum of the compound does not contain the absorption bands characteristic of such a structure (I), which is, therefore, very unlikely.*

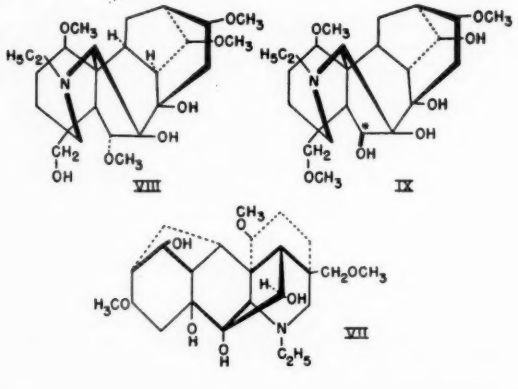
[4]*The high melting point of V and the shift in carbonyl frequency in its infrared spectrum may be due to considerable hydrogen bonding. In fact, the infrared showed a strongly bonded* NH.

$C_{24}H_{35}O_7N$ (VI) (infrared band at 1752 cm.$^{-1}$), still containing the internal ether (infrared bands at 1005 and 900 cm.$^{-1}$), which, therefore, must be anhydrohydroxy-dehydrodelcosine. Compound VI proved to be identical with a product obtained in minute quantity in the oxidation of delcosine with chromium trioxide – pyridine complex (9). It tended to form a hydrate and was difficult to extract.

In order to investigate whether the hydroxyl group oxidized by chromic acid or by the Oppenauer oxidation to give the ketone I was the same as that involved in the formation of compound V in the oxidation of IV, attempts were made to reduce selectively the ether system. For this purpose the ethylated derivative VI was used, but all attempts proved unsuccessful. An indication that the hydroxyl involved in both cases was the same can be obtained by a comparison of the pK_a values of the various compounds. The decrease in pK_a in going from delcosine (pK_a 6.49) to dehydrodelcosine I (pK_a 5.75) and from N-desethyl-anhydrohydroxydelcosine (IV) (pK_a 4.6) to the corresponding ketone V (pK_a 4.0) is small in both cases, i.e., 0.74 and 0.6 units respectively. The hydroxyl, therefore, does not seem to have been in close proximity to the nitrogen since Cookson and Trevett (3) have shown that in delpheline (pK_a 7.6) oxidation of a hydroxyl close to the nitrogen to give dehydrodelpheline (pK_a 5.5) caused a decrease in pK_a of 2.1 units.

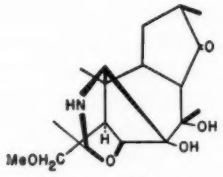
Silver acetate oxidation of delcosine gave anhydrohydroxydelcosine III together with some N-desethyldelcosine (II). Oxidation with mercuric acetate in acetic acid gave a mixture of N-desethyl-anhydrohydroxydelcosine (IV), N-desethyldelcosine (II), and a compound which was very soluble in water and was not isolated. The aqueous solution containing this compound was acidified and some of the water distilled off. The distillate contained acetaldehyde which was isolated as its *p*-nitrophenylhydrazone. The remaining acid aqueous solution was rendered basic with ammonia and on extraction yielded a further quantity of N-desethyldelcosine II. The water-soluble compound which could not be isolated as such must, therefore, have been the carbinolamine of delcosine formed by hydroxylation of the methylene in the ethyl group which was subsequently split by acid to N-desethyldelcosine and acetaldehyde. A similar oxidation in delpheline was observed by Cookson and Trevett (3).

Delcosine ($C_{24}H_{39}O_7N$), lycoctonine ($C_{25}H_{41}O_7N$), and delpheline ($C_{25}H_{39}O_6N$) are three *Delphinium* alkaloids, and the first two occur together in some species. If their substituents be replaced by hydrogen, all three can be represented by the common saturated empirical formula $C_{21}H_{33}N$, which is that of a hexacyclic structure. It is, therefore, legitimate to assume that the three alkaloids possess closely related structures.



The chemical investigation of lycoctonine has led to a partial elucidation of its structure (5) while an X-ray crystallographic analysis has made it possible to advance a total structure VII (10) which can also be written in the diterpenoid form VIII.[5] In order to accommodate the chemistry of delpheline which parallels that of lycoctonine, Cookson and Trevett (3) have recently suggested the same ring structure for delpheline in which, however, some of the substituents are different although similarly located. The behavior of delcosine towards oxidizing agents described above and the acylation results described previously (12) can be rationalized by assuming for delcosine a structure IX with the same ring arrangement as that proposed for lycoctonine. The difference between delcosine, lycoctonine, and delpheline would then rest with the oxygenated substituents and with the different orientation of the substituent at the asterisked position in IX.

To account for the formation of N-desethyl-anhydrohydroxy-dehydrodelcosine V, which contains both an internal ether and a five-membered ketone, it is necessary that the substituent on the five-membered ring remote from the nitrogen should be a secondary hydroxyl. Also that a hydroxyl be present in the pentatomic ring adjacent to the heterocyclic ring instead of the methoxyl present in lycoctonine, and that its orientation be opposite to that obtaining in lycoctonine. It is this particular orientation of the hydroxyl that accounts for the formation of an internal ether instead of a lactam. Lycoctonine (4) and delpheline (2) both form lactams on oxidation, and the latter contains a hydroxyl in the same position as that marked with an asterisk in IX. This hydroxyl in delpheline just like the methoxyl in lycoctonine must, therefore, be oriented away from the α-position to the nitrogen. On the basis of structure IX the compound V (N-desethyl-anhydrohydroxy-dehydrodelcosine) can be represented as shown by the partial formula V, which also shows the internal ether.



V

In the course of the isolation of the various oxidation products described, no acidic material was isolated, and it is concluded that no primary alcoholic group is present in the alkaloid. The presence of a vicinal triol system in delcosine should be detectable by oxidation with periodate, but this oxidation gave anomalous results, and up to 5 moles of oxidant were consumed in 120 hours. On the other hand, anhydrohydroxydelcosine (in which one of the hydroxyls is bound in an internal ether) on similar oxidation consumed 1 mole of periodate, thus indicating the presence of vicinal hydroxyls in that compound. The results of the periodate cleavage studies will be reported later.

Although the size of the heterocyclic ring, and many features paralleling lycoctonine chemistry still have to be proved, we tentatively propose to represent delcosine by structure IX on the basis of which all the known facts can be rationalized.

[5]*We feel that the representation VII of the structure of lycoctonine used heretofore by this group is a better illustration of the spatial model produced by X-ray crystallography than the diterpenoid representation. Further, the diterpenoid formula gives a distorted aspect to the rings, and does not lend itself as well to the illustration of the various rearrangements undergone by lycoctonine. Since, however, most of the workers in this field of alkaloid chemistry are using the diterpenoid representation, we are adopting it in spite of its shortcomings, in order to avoid confusion in the literature.*

EXPERIMENTAL

All the infrared spectra were measured in nujol mulls (unless otherwise mentioned) on a Perkin-Elmer double-beam spectrometer model 21B, using a sodium chloride prism. The specific rotations were measured in chloroform solutions (accuracy ±1.5°) and the pK_a were determined in 50% ethanol by electrometric titration.

Oxidation of Delcosine with Chromic Acid in Acetic Acid (Dehydrodelcosine I)

To a solution of delcosine (1.35 g.) in glacial acetic acid (10 ml.) chromium trioxide (0.505 g.) was added portionwise. The solution was set aside for 24 hours at room temperature, and then refluxed for 90 minutes. The solvent was removed under reduced pressure and the residue partitioned between dilute sulphuric acid and chloroform (100 ml.). The chloroform layer was washed with a fresh quantity of dilute acid, and the combined acid extract and washings brought to pH 10 with aqueous 40% sodium hydroxide. This alkaline solution was extracted with chloroform and the extract on evaporation left 0.926 g. of a gummy basic product. The chloroform layer separated from the dilute sulphuric acid solution yielded acidic and neutral material (0.139 g.) but no crystalline product could be obtained from this fraction.

The gummy basic product was dissolved in benzene and chromatographed on a column of grade V alumina. Elution with the first 100 ml. of benzene gave a gum, whereas the next 100 ml. gave a partially crystalline material (259 mg.). This was recrystallized from ethanol, m.p. 199–200°. Mixture with delcosine depressed its melting point. Its infrared absorption spectrum in nujol showed a band at 1755 $cm.^{-1}$. The further eluates from the chromatogram yielded unchanged delcosine.

Paper chromatography, by the descending technique on Whatman No. 1 paper buffered to pH 4.78 (8) with butanol saturated with water as solvent, showed that the crystalline product consisted of a mixture of delcosine (R_f 0.546) and a ketonic substance (R_f 0.725). The spots were made visible with Dragendorff's reagent.

In a second experiment, delcosine (6.75 g.) dissolved in acetic acid (50 ml.) was oxidized with chromic acid (2.525 g.) at room temperature. After the products were worked up as above, the acid fraction obtained weighed 1.07 g. It was amorphous, and chromatography on alumina or celite failed to yield a crystalline substance. On the other hand, the basic fraction (4.406 g.) when chromatographed on grade II alumina gave 1.23 g. of a mixture of gummy and crystalline material. This mixture when chromatographed again on alumina and eluted with 300 ml. of benzene containing 20% of chloroform yielded the ketonic product (0.357 g.). This was crystallized from a mixture of benzene and petroleum ether from which it separated as colorless prisms. Five recrystallizations from dilute ethanol furnished an analytical sample of dehydrodelcosine, m.p. 212.5–213.5°. Found: C, 63.58; H, 8.10. Calc. for $C_{24}H_{37}O_7N$: C, 63.83; H, 8.26%. The pK_a was 5.75 and the molecular weight 457 (calc. 451). The infrared absorption spectrum contained a strong band at 1755 $cm.^{-1}$. A paper chromatogram of the pure dehydrodelcosine gave one spot (R_f 0.725).

In a later experiment, it was found more convenient to purify the ketone by countercurrent distribution. The crude mixture of basic products (3.0 g.) was distributed in 12 transfers between benzene (100 ml. in each tube) and a buffer (100 ml.) pH 4.92, made by mixing 55 volumes of *M* dipotassium acid phosphate and 45 volumes of 0.5 *M* citric acid. When the distribution was complete, the aqueous solutions were made alkaline with aqueous 40% sodium hydroxide (13 ml.) and the organic solutes driven into

the benzene layers. Delcosine (1.52 g.) was contained in tubes 11 and 12 whereas dehydrodelcosine was found in tubes 7, 8, and 9 (568 mg.). No crystalline product was isolated from the other tubes although a peak occurred in tube 3.

Dehydrodelcosine Oxime

Hydroxylamine hydrochloride (58 mg.) and dehydrodelcosine (95 mg.) were dissolved in ethanol, and the pH of the solution brought to 9 by addition of 10% sodium hydroxide. After the solution had refluxed for 3.5 hours the solvent was evaporated under reduced pressure and the residue dissolved in water. The pH was adjusted to 7 and the solution extracted with chloroform. Evaporation of the chloroform extract gave the crystalline oxime, which, after recrystallization from ethanol, consisted of colorless needles, m.p. 229.5–230.5°. Found: C, 62.03; H, 8.44; N, 6.09. Calc. for $C_{24}H_{38}O_7N_2$: C, 61.78; H, 8.21; N, 6.00%. The infrared absorption spectrum contained no band in the carbonyl region.

Oppenauer Oxidation of Delcosine

A solution of aluminum isopropoxide (7.26 g.) in dry benzene (100 ml.) was added in the course of 30 minutes to a refluxing solution of delcosine (1.35 g.) in dry benzene (60 ml.) containing cyclohexane (40 ml.). The mixture was refluxed for 4 hours, left overnight at room temperature, and refluxed for a further 5 hours. It was acidified with glacial acetic acid and steam distilled to remove the volatile solvents. After cooling, the residual aqueous solution was brought to pH 1 with 3 *N* sulphuric acid and extracted with chloroform. The pH was then adjusted to 10 by the addition of 40% sodium hydroxide and the solution extracted with chloroform. This chloroform extract, on evaporation, left a gummy residue, 1.24 g., which was dissolved in ethanol. On cooling, delcosine (0.33 g.) crystallized. The residual basic material recovered from the mother liquor was dissolved in benzene and chromatographed on grade V alumina. The eluate yielded the mixture of delcosine and dehydrodelcosine, m.p. 199–200°, wt. 181 mg., previously described.

Reduction of Dehydrodelcosine

Dehydrodelcosine (10 mg.) and sodium borohydride (30 mg.) were added to methanol containing a few drops of water (3 ml.) and the mixture left at room temperature for 15 minutes. The solution was then heated on the steam bath for 1 hour, diluted, and allowed to cool. Colorless, glistening hexagons separated out, m.p. 201–203°, either alone or mixed with delcosine.

Silver Oxide Oxidation of Delcosine

Freshly prepared silver oxide (from 7.4 g. of silver nitrate) was added to a solution of delcosine (2.86 g.) in ethanol (150 ml.) and water (150 ml.). The mixture was kept at 80° with vigorous stirring for 68 hours, then cooled and filtered. The residue of metallic silver and silver oxide was washed well with ethanol and the combined filtrate and washings was evaporated to a sirup under reduced pressure. This sirup was partitioned between 3 *N* sulphuric acid and chloroform, and the aqueous layer separated and extracted with three 50 ml. portions of chloroform. The combined chloroform extract was back extracted with 3 *N* sulphuric acid, washed with water, and evaporated to dryness. It left a brown residue (30 mg.) which was not further investigated.

The combined acid liquor and washings was made alkaline with ammonia and extracted with chloroform. Evaporation of the chloroform extract gave a colorless residue (2.326 g.). This, on trituration with benzene (5 ml.), left a crystalline compound (0.679 g.), m.p. 230–232°, which, after recrystallization from ethanol (charcoal), melted at 240–241° (dec.) (N-desethyldelcosine II), pK_a 7.9, $[\alpha]_D^{22}$ +79.5° (*c*, 0.88). Found: C, 62.13; H, 8.24; N, 3.36; act. H (in dioxane), 1.05. Calc. for $C_{22}H_{35}O_7N$: C, 62.09; H, 8.29; N, 3.29; 5 act. H, 1.18%.

The benzene liquor left after filtration of N-desethyldelcosine was diluted with benzene and chromatographed on grade V alumina. It was eluted with benzene and the first 125 ml. of eluate contained a base, 0.602 g., m.p. 187–188°. Further elution with benzene (325 ml.) and then with benzene containing 1% ethanol (100 ml.) yielded a further 0.258 g. of the same product. Continued elution with benzene containing 1% ethanol (300 ml.) gave N-desethyldelcosine, 0.155 g., m.p. 240–241° (dec.).

The product melting at 187–188° (anhydrohydroxydelcosine III) had pK_a 4.2 and $[\alpha]_D^{22}$ +74.6° (*c*, 1.4). Found: C, 63.92; H, 8.04; N, 3.35; act. H, 0.56. Calc. for $C_{24}H_{37}O_7N$: C, 63.83; H, 8.26; N, 3.10; 3 act. H, 0.66%. Its infrared absorption spectrum showed no carbonyl nor double bond absorption but contained two sharp bands at 1000 and 900 cm.$^{-1}$.

Anhydrohydroxydelcosine Perchlorate

A solution of anhydrohydroxydelcosine (161 mg.) in methanol (5 ml.) was made just acid by the cautious addition of 70% perchloric acid. Dropwise addition of ethyl acetate with cooling and stirring caused the salt to separate as colorless needles (141 mg.). Recrystallized from methanol–ether containing a trace of perchloric acid, it melted at 216–217° (dec.). Found: C, 51.94; H, 7.07; N, 2.52. Calc. for $C_{24}H_{37}O_7N.HClO_4$: C, 51.90; H, 6.90; N, 2.52%. The infrared absorption spectrum of the salt contained no absorption bands in the region 1550–1800 cm.$^{-1}$. The perchlorate is therefore not an anhydronium salt.

Ethylation of N-Desethyldelcosine

N-Desethyldelcosine (35 mg.), sodium carbonate (39 mg.), and dimethylformamide (250 mg.) containing ethyl iodide (79 mg.) were heated on the steam bath for 6.5 hours. The solution was cooled, acidified with dilute sulphuric acid, and extracted with chloroform. It was then rendered basic with ammonia and again extracted with chloroform. This second extract, on evaporation, gave colorless rhombs, m.p. 197–202°, which on recrystallization from dilute methanol, separated as hexagons, m.p. 201–202°, either alone or mixed with delcosine.

Acetylation of N-Desethyldelcosine

A mixture of N-desethyldelcosine (141 mg.), pyridine (two drops), and acetic anhydride (2.5 ml.) was left at room temperature for 20 hours. A sufficient quantity (15 ml.) of aqueous sodium carbonate to produce a basic solution[6] was added. Extraction with chloroform gave a colorless gum (153 mg.) that could not be crystallized, and was possibly a mixture of O,N-diacetate and O,O,N-triacetate. It was hydrolyzed with aqueous sodium carbonate on the steam bath for 5 hours and, after cooling, the solution was extracted with chloroform. The extract, on evaporation, left a crystalline residue, m.p. 221–225°, which in admixture with N-desethyldelcosine melted at 190–220°.

[6]*It is important to keep the aqueous solutions basic throughout, otherwise the product cannot be crystallized.*

N-Acetyl-N-desethyldelcosine, after crystallization from acetone, consisted of colorless needles, m.p. 221–225°. Found: C, 61.78; H, 8.17; N, 3.07. Calc. for $C_{24}H_{37}O_8N$: C, 61.65; H, 7.98; N, 3.00%. The infrared absorption spectrum showed no band at 1730 cm.$^{-1}$ characteristic of an acetoxy group, but contained a band at 1643 cm.$^{-1}$ characteristic of an amide carbonyl.

Hydrolysis of N-Acetyl-N-desethyldelcosine

The N-acetyl derivative (50 mg.) was hydrolyzed by being heated on the steam bath with 20% aqueous potassium hydroxide for 2 hours. Extraction of the cooled solution with chloroform gave N-desethyldelcosine, m.p. 239–241°, either alone or mixed with an authentic sample.

Reduction of Anhydrohydroxydelcosine

(*a*) Anhydrohydroxydelcosine (258 mg.) was dissolved in 50% aqueous methanol (5 ml.), and sodium borohydride (50 mg.) was added to the solution. The solution was left standing at room temperature for 18 hours, and subsequently heated on the steam bath for 1 hour. After cooling, the solution was extracted with chloroform, and the extract, on evaporation, left a residual white powder, m.p. 196–200°, wt. 236 mg. This was purified by chromatography on alumina and crystallized from dilute methanol from which it separated as colorless hexagons, m.p. 201–203° either alone or mixed with delcosine.

(*b*) Anhydrohydroxydelcosine (54.6 mg.) was hydrogenated in aqueous perchloric acid in the presence of Adams' catalyst (33 mg.) at atmospheric pressure and room temperature (26°). One mole of hydrogen was absorbed smoothly in 30 minutes, but no further uptake of hydrogen was observed within the next 2 hours. The solution was diluted with water and the catalyst filtered and washed with dilute ethanol. The combined filtrate and washings was made alkaline with ammonia and extracted with chloroform. Evaporation of the extract left a crystalline residue (45 mg.), m.p. 199–201° and, in admixture with delcosine, m.p. 200–203°.

Oxidation of Delcosine with N-Bromosuccinimide

Delcosine (500 mg.) was dissolved in aqueous acetone (4 acetone:1 water, 10 ml.) containing a few drops of glacial acetic acid. Sodium acetate (450 mg.) and N-bromosuccinimide (1.75 equivalents) were added at room temperature. On addition of the latter the solution turned yellow, and the color disappeared in 30 seconds as some heat was evolved. The solution was diluted with water (30 ml.) and allowed to stand at room temperature for 20 hours. Dilute sulphuric acid was added to bring the solution to pH 2, and the acidic and neutral material was removed by extraction with chloroform. The aqueous solution was then made alkaline with ammonia and again extracted with chloroform. The basic product (350 mg.) left after evaporation of this chloroform extract was dissolved in benzene and chromatographed on grade V alumina. Elution with benzene–ether (1:1) gave anhydrohydroxydelcosine III (197 mg.), which crystallized from acetone–petroleum ether as colorless prisms, m.p. 188–189°, identical with the compound obtained from the silver oxide oxidation. Further elution with benzene containing 10% ethanol gave N-desethyldelcosine II (138 mg.), which after crystallization from ethanol melted at 239–240°. Mixture with N-desethyldelcosine obtained in the silver oxide oxidation failed to alter the melting point, and comparison of the infrared absorption spectra confirmed the identity.

In a second experiment, delcosine (578 mg.) was dissolved in acetone and oxidized with N-bromosuccinimide (358 mg.) in the presence of sodium carbonate (480 mg.). The solution (pH 8–9) was diluted with water and left at room temperature for 20 hours. When worked up as above, anhydrohydroxydelcosine (80 g.) was obtained. The N-desethyldelcosine fraction, however, was accompanied by a compound separating as needles which was very soluble in acetone. It was readily separated from N-desethyldelcosine, which is sparingly soluble in acetone. The new product crystallized from acetone–petroleum ether as needles, m.p. 218–219°, which proved to be N-desethyl-anhydrohydroxydelcosine IV. It had pK_a 4.6 and $[\alpha]_D^{22}$ +132° (c = 0.75). Found: C, 62.60, 62.47; H, 7.82, 7.97; N, 3.85. Calc. for $C_{22}H_{33}O_7N$: C, 62.39; H, 7.85; N, 3.31%.

Ethylation of N-Desethyl-anhydrohydroxydelcosine

N-Desethyl-anhydrohydroxydelcosine (70 mg.) was added to a solution of sodium carbonate in freshly distilled ethyl iodide (275 mg.) and dimethylformamide (350 mg.) and heated on the steam bath for 5 hours. The solution was cooled overnight, during which silky needles were deposited. The crystalline hydriodide was dissolved in water, and the solution made basic with sodium hydroxide and extracted with chloroform. The product obtained from the extract consisted of anhydrohydroxydelcosine (50 mg.), which after purification by chromatography melted at 187–188° either alone or in admixture with an authentic sample. Comparison of the infrared absorption spectra which were superimposable confirmed the identity.

Reduction of N-Desethyl-anhydrohydroxydelcosine

The compound (88 mg.) was dissolved in 50% aqueous methanol and sodium borohydride (23 mg.) was added to the solution which was kept at room temperature for 20 hours, subsequently heated on the steam bath for 10 minutes, and allowed to cool. On standing, crystals of N-desethyldelcosine (20 mg.) separated. Chloroform extraction of the mother liquor yielded a further 35 mg. of the same compound. After recrystallization the melting point was raised to 240–241° and was undepressed by mixing with an authentic sample of N-desethyldelcosine.

Oxidation of N-Desethyl-anhydrohydroxydelcosine with Potassium Permanganate

N-Desethyl-anhydrohydroxydelcosine (194 mg.) was dissolved in 5% aqueous potassium hydroxide (10 ml.) and a 1% solution of potassium permanganate in 5% aqueous potassium hydroxide (30 ml.) was added gradually over an hour at room temperature. The solution was then diluted and warmed on the steam bath to complete the reaction. The manganese dioxide was filtered off and the excess permanganate reduced with a concentrated solution of sodium sulphite. The liquor was then extracted with chloroform and the extract evaporated to dryness. There was left a crystalline residue (122 mg.), m.p. 275–293°. Repeated recrystallization from acetone–petroleum ether and from ether – petroleum ether raised the melting point to 298°. Its pK_a was 4.0. Found: C, 62.61; H, 7.99; N, 3.48. Calc. for $C_{22}H_{31}O_7N$: C, 62.69; H, 7.41; N, 3.32%. The analytical figures agree with those required by N-desethyl-anhydrohydroxy-dehydrodelcosine and the infrared absorption spectrum contained the two bands at 1000 cm.$^{-1}$ and 900 cm.$^{-1}$ typical of the internal ether and a sharp band at 1743 cm.$^{-1}$ indicating a five-membered cyclic ketone.

Reduction of N-Desethyl-anhydrohydroxy-dehydrodelcosine

The keto compound (12 mg.) was reduced with sodium borohydride by heating (12 mg.) in aqueous methanol on the steam bath for 1 hour. Chloroform extraction of

the reaction mixture gave the product (11 mg.) as needles, m.p. 213–215°. Recrystallized from acetone–petroleum ether, it consisted of colorless needles, m.p. 216–218°, either alone or in admixture with N-desethyl-anhydrohydroxydelcosine.

Ethylation of N-Desethyl-anhydrohydroxy-dehydrodelcosine

A mixture of N-desethyl-anhydrohydroxy-dehydrodelcosine (50 mg.), ethyl iodide (1 ml.), dimethylformamide (20 mg.), and sodium carbonate (20 mg.) was heated on the steam bath for 5 hours. The reaction mixture was evaporated to dryness, and the residue triturated with hot dry acetone and filtered. The filtrate was evaporated to dryness, the residue taken up in dry benzene containing a little acetone, and the solution filtered and evaporated to dryness. The residual anhydrohydroxy-dehydrodelcosine was crystallized from alcohol–ether from which it separated as needles, m.p. 192–193°. $[\alpha]_D^{23}$ +34.9° (c = 1.46). Found: C, 63.95; H, 7.95; N, 3.14. Calc. for $C_{24}H_{35}O_7N$: C, 64.12; H, 7.85; N, 3.12%.

Oxidation of Delcosine with Potassium Permanganate[7]

Delcosine (231 mg.) was dissolved in N potassium hydroxide and the solution cooled to 0°. A solution of 1% of potassium permanganate in N potassium hydroxide was added dropwise to the stirred solution of delcosine. The addition was continued until the permanganate color persisted and during the addition the temperature was allowed to rise to room temperature. The excess permanganate was destroyed with sodium sulphite, the solution filtered, and the filtrate extracted with chloroform. Evaporation of the extract left a crystallized residue (65 mg.), m.p. 210–280°. Fractional crystallization from a mixture of chloroform, ether, and petroleum ether gave a compound separating as needles (44 mg.), m.p. 280–285°, that proved to be identical with N-desethyl-anhydrohydroxy-dehydrodelcosine (see above), and also a smaller quantity (17 mg.) of N-desethyl-anhydrohydroxydelcosine, m.p. 218°.

Oxidation of Delcosine with Silver Acetate

Delcosine (405 mg.) and silver acetate (1.445 g.) were refluxed in 3% acetic acid (50 ml.) for 40 minutes. The hot solution was filtered from the metallic silver, and filtered again, after cooling, from the silver acetate that had crystallized. Dilute sulphuric acid was added to the filtrate and the acid solution extracted with chloroform. The aqueous solution was then made alkaline with ammonia and again extracted with chloroform. The gum left after evaporation of the chloroform was dissolved in benzene and chromatographed as usual. It yielded anhydrohydroxydelcosine (230 mg.), m.p. 187–188°, undepressed by mixture with the sample obtained by silver oxide oxidation. No other pure product could be isolated from the reaction.

Oxidation of Delcosine with Mercuric Acetate

Delcosine (3.0 g.) was dissolved in 3% acetic acid (65 ml.), and mercuric acetate (16.8 g.) was added. The solution was heated on the steam bath for 60 minutes. The precipitated mercurous acetate was filtered off and the filtrate extracted with chloroform. Evaporation of the chloroform extract left a mixture of N-desethyldelcosine and N-desethyl-anhydrohydroxydelcosine which was directly reduced with sodium borohydride to give N-desethyldelcosine (1.9 g.). The aqueous solution that had been extracted was acidified with dilute hydrochloric acid, and an aliquot (25 ml.) distilled at atmospheric pressure to about half volume. The aqueous distillate was treated with an acetic acid solution of p-nitrophenylhydrazine containing a few drops of hydrochloric acid. Yellowish

[7] *This oxidation was first carried out by Dr. W. E. Walles in this laboratory.*

brown needles separated, m.p. 127–130.5°. Recrystallization from aqueous methanol raised the melting point to 130–130.5°, and this was undepressed by mixing with an authentic sample of acetaldehyde *p*-nitrophenylhydrazone.

The acidic solution from which the aliquot had been taken was hydrolyzed on the steam bath, cooled, made alkaline with ammonia, and extracted with chloroform. The extract on evaporation yielded a further quantity of N-desethyldelcosine.

ACKNOWLEDGMENT

The authors wish to express their indebtedness to Dr. O. E. Edwards for many valuable discussions, and to Mr. R. Lauzon for taking the infrared absorption spectra.

REFERENCES

1. BERGMANN, E. and PINCHAS, S. Rec. trav. chim. **71**, 161 (1952).
2. COOKSON, R. C. and TREVETT, M. E. Chemistry & Industry, 1324 (1954).
3. COOKSON, R. C. and TREVETT, M. E. J. Chem. Soc. 3121 (1956).
4. EDWARDS, O. E. and MARION, L. Can. J. Chem. **30**, 627 (1952).
5. EDWARDS, O. E. and MARION, L. Can. J. Chem. **32**, 195 (1954).
6. FIESER, L. F. and RAJAGOPALAN, S. J. Am. Chem. Soc. **71**, 3938 (1949).
7. GOODSON, J. A. J. Chem. Soc. **245** (1945).
8. MUNIER, R. Bull. soc. chim. biol. **35**, 1225 (1953).
9. POOS, G. I., ARTH, G. E., BEYLER, R. E., and SARETT, L. H. J. Am. Chem. Soc. **75**, 422 (1953).
10. PRZYBYLSKA, M. and MARION, L. Can. J. Chem. **34**, 185 (1956).
11. SCHÖPF, C. and KLEIN, D. Chem. Ber. **87**, 1638 (1954).
12. TAYLOR, W. I., WALLES, W. E., and MARION, L. Can. J. Chem. **32**, 780 (1954).
13. WOODWARD, R. B., WENDLER, N. L., and BRUTSCHY, F. J. J. Am. Chem. Soc. **67**, 1425 (1945).

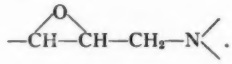
THE STRUCTURE OF ANNOTININE[1]

M. MARTIN-SMITH,[2] R. GREENHALGH,[2] AND LÉO MARION

ABSTRACT

Annotinine is hydrolyzed by aqueous barium hydroxide to annotininic acid, $C_{16}H_{23}O_4N$, which with diazomethane forms methyl annotininate. Treatment of this ester (or of annotinine) with potassium methoxide in methanol produces epimerization and gives rise to methyl epiannotininate, also produced by methanolysis of annotinine. Whereas under the action of barium hydroxide methyl annotininate regenerates annotininic acid, methyl epiannotininate is converted to annotinine hydrate. Hydrolysis of annotinine with dilute sulphuric acid gives rise to annotinine diol, which is oxidizable to an amino acid, whereas the isomeric annotinine hydrate on oxidation gives rise to a hydroxyketo lactone. This corroborates the evidence already in the literature indicating that the γ-lactone ring in annotinine hydrate is not the same as that originally present in the alkaloid. The relevant chemical reactions of annotinine are reviewed in the light of a tentative structure suggested for annotinine.

It has been established previously that annotinine ($C_{16}H_{21}O_3N$) is a tertiary base containing a γ-lactone and a cyclic ether (6) that MacLean and Prime (5) have later shown to be an epoxide. The same authors have also established the position of the epoxide system relative to the nitrogen as



Annotinine reacts with alcoholic alkali giving rise to annotinine hydrate (6), a substance containing a γ-lactone and two hydroxyls. Henderson, Stonner, Valenta, and Wiesner (4) were the first to suggest that the γ-lactone of annotinine hydrate was not that originally present in the alkaloid, but a new γ-lactone formed by relactonization of the carboxyl with one of the hydroxy groups originating from the oxide ring. Wiesner and his collaborators (8) by a study of the reactions of annotinine anhydrochlorolactam, formed by dehydration of the lactam chlorohydrin, have supplied evidence supporting such a suggestion. The same authors have also suggested a number of tentative structural formulae for the alkaloid.

In this paper we wish to record a number of experiments supplying more evidence pointing to a different relactonization in the formation of annotinine hydrate, and also to suggest a tentative structure for the alkaloid that seems to accommodate all the chemical evidence.

It was reported by Henderson, Stonner, Valenta, and Wiesner (4) that annotinine on prolonged treatment with boiling dilute sulphuric acid led to a derivative, m.p. 75°, whose empirical formula $C_{16}H_{23}O_4N$ differed from that of annotinine by the addition of the elements of water. Careful repetition of this work failed in our hands to give a compound of that melting point, but gave rise to three products. One was present only in traces while the other two were annotinine hydrate (8%) and the isomeric annotinine diol, m.p. 190–192°. This last compound formed a diacetyl derivative, m.p. 168°, which is obviously the same as that prepared by Henderson, Stonner, Valenta, and Wiesner (4) from their material. It is probable that annotinine diol is a substance in which the γ-lactone is the same as that present in the alkaloid.

[1]*Manuscript received January 29, 1957.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 4275.
[2]*National Research Council of Canada Postdoctorate Fellow.*

When annotinine was boiled for several hours with aqueous barium hydroxide it gave rise to a trihydroxy acid which on treatment with 25% sulphuric acid was also converted to annotinine diol. Oxidation of this diol with barium permanganate gave rise to the same amino acid, $C_{14}H_{19}O_4N$, previously obtained from "unsaturated lactone A" (see below) (1) and from annotinine anhydrochlorolactam (4). On the other hand, oxidation of annotinine hydrate has been shown (7) to give rise to a hydroxyketo lactone ($C_{16}H_{21}O_4N$).

When annotinine was treated with boiling aqueous barium hydroxide for 40 minutes, there resulted a good yield of a hydroxy acid, $C_{16}H_{23}O_4N$. It is proposed to designate this acid, which still contains the epoxide ring intact, annotininic acid. The infrared spectrum of the acid did not show absorption at a normal carboxyl frequency but instead contained a diffuse peak at 1575–1568 cm.$^{-1}$. Such absorption is typical of an amino acid existing as a zwitterion. On treatment with diazomethane, annotininic acid was smoothly converted to its methyl ester, m.p. 132.5–134°. Boiling methyl annotininate with aqueous barium hydroxide regenerated annotininic acid. Treatment of methyl annotininate with potassium methoxide in absolute methanol converted it into an isomeric methyl ester, m.p. 163–164°. This ester (methyl epiannotininate) also resulted when annotinine was treated with an equivalent of potassium methoxide in methanol at room temperature. The action of barium hydroxide on this ester converted it to annotinine hydrate in contrast with the action of the same reagent on methyl annotininate, which regenerated the acid.

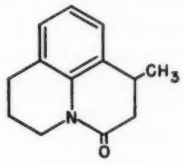
Methyl epiannotininate had previously been reported by Henderson, Stonner, Valenta, and Wiesner (4), who prepared it by the action of methanolic potassium hydroxide on annotinine, followed by methylation with diazomethane. This sequence masked the essential nature of the reaction, which is a methanolysis of the lactone accompanied by an epimerization. Such a transesterification had been observed with the naturally occurring lactones picrotin and picrotoxinin (3) under the influence of potassium hydroxide in absolute methanol (such a solution containing a high concentration of methoxide ions). Further, it seems highly improbable that epiannotininic acid could be isolated as such since it passes extremely readily into annotinine hydrate.

A similar transesterification with epimerization was observed by Wiesner and collaborators (4, 8) when methanolic potassium hydroxide was allowed to react with unsaturated lactamic derivatives of annotinine.

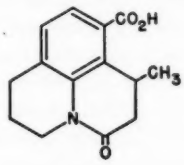
Annotinine lactam itself underwent the same transesterification to form methyl epiannotininate lactam, which was also obtained by oxidation of methyl epiannotininate.

It has been shown by MacLean and Prime (5) that the lactam obtainable from annotinine can be converted to a lactam chlorohydrin ($C_{16}H_{20}O_4NCl$), which in turn can be dehydrated to an anhydrochlorolactam ($C_{16}H_{18}O_3NCl$). Later, Henderson, Stonner, Valenta, and Wiesner (4) described the oxidation of this last compound to an amino acid, $C_{14}H_{19}O_4N$, still containing a γ-lactone ring, which must be that initially present in the alkaloid. Subsequently, Anet and Marion (1) showed that this same amino acid could be obtained by oxidation of the "unsaturated lactam A" produced by mild treatment of annotinine chlorohydrin with chromous chloride (7). The methyl ester of this amino acid underwent transesterification under the influence of potassium methoxide and gave rise to a diester. Anet and Marion (1) have also shown that in the presence of palladium at 250°, the amino acid is readily dehydrogenated to an acid, $C_{14}H_{15}O_3N$, that no longer contains a lactone ring, but contains a lactamic carbonyl as well as a free carboxylic group. Furthermore, this acid can be decarboxylated to a neutral compound,

$C_{13}H_{15}ON$. The structure originally advanced for the neutral compound (1) is erroneous and Valenta, Wiesner, Bankiewicz, Henderson, and Little (9) have established its structure and that of the lactam acid by synthesis to be I and II respectively.



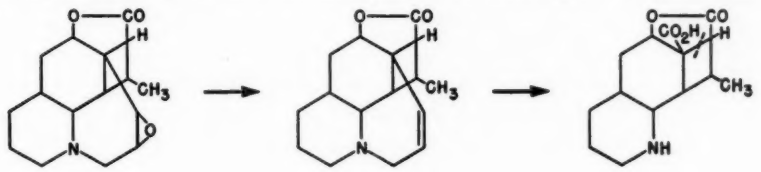
I



II

None of the sequence of reactions involved in going from annotinine to the acid II and the neutral compound I is of a drastic nature, with the possible exception of the palladium dehydrogenation. Since, however, the product of this dehydrogenation has retained all its atoms, and its asymmetric center has retained its optical activity, it seems legitimate to assume that no deep-seated rearrangement can be involved in the formation of these compounds and of the amino acid. Any structure suggested for annotinine should, therefore, be such as to permit of the easy formation of these derivatives. It should contain a $C—CH_3$ group and also account for the formation of 8-*n*-propylquinoline (2) and of 7-methylquinoline (1), which were obtained in small yield from the alkaloid under dehydrogenating conditions. It should contain an epoxide ring (5, 6) and a γ-lactone with the carboxyl so located as to be capable of relactonization to a new γ-lactone with one of the hydroxyl groups obtainable by hydrolysis of the epoxide.

We now wish to suggest the tentative structure III for annotinine. It contains the requisites mentioned above, and supplies a basis for all the chemical transformations of annotinine so far observed. On the basis of this structure the "unsaturated lactone A" would be represented by IV and the oxidation of IV would give V, which must be the

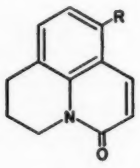


III IV V

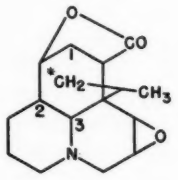
structure of the amino acid $C_{14}H_{19}O_4N$. The passage from V to the lactam acid II on heating in the presence of palladium would involve, first, the rupture of the O—C bond, then rupture of the bond that is marked in the four-membered ring, aromatization of the six-membered carbon ring, and lactamization of the liberated carboxyl with the secondary amino group. Methyl annotininate and methyl epiannotininate would be represented by partial formulae VI and VII respectively.

VI VII

In two short communications Wiesner and his co-workers (10, 11) have recently reported that the hydroxy ester of annotinine $C_{17}H_{22}O_4NCl$ (obtained by methanolysis of anhydrochloro annotinine lactam), when refluxed in xylene with phosphorus pentoxide, gave rise to two isomeric esters $C_{17}H_{20}O_3NCl$. One was simply an anhydro product while the other (isolated as the free acid) showed the spectroscopic features of an α-pyridone (10), and had undergone scission in one ring with formation of a double bond (11). The anhydro ester product when heated in the presence of palladium–charcoal produced a quinolone acid VIII (R = CO_2H) which could be decarboxylated to the quinolone VIII (R = H). Wiesner, Valenta, Ayer, and Bankiewicz (11) consider that the production of these quinolone derivatives limits the structure of annotinine to IX in which an additional connection has to be made between *C and C(1), C(2), or C(3), of which they deem a *C—C(2) bond preferred.



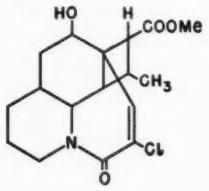
VIII



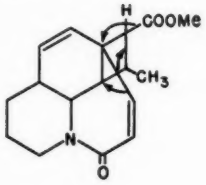
IX

We consider that our tentative formula III permits the rationalization of these reactions equally well and furthermore makes the derivation of compounds such as I and II much more obvious than does structure IX.

On the assumption of structure III the hydroxy epiester derived from the anhydrochlorolactam can be represented by X and the anhydro ester by XI. A concerted rearrange-



X



XI

ment involving a contraction of the seven-membered ring in XI, a wandering of a carboxyl group, and aromatization and elimination of the side chain results in the formation of VIII (R = CO_2H).

EXPERIMENTAL

The infrared absorption spectra were taken in nujol mulls with a Perkin-Elmer double beam spectrometer Model 21B with a sodium chloride prism.

Hydroxylactone and "Unsaturated Lactone A"

The preparation described previously (7) was not entirely satisfactory and the following procedure gave better yields of the two compounds. Annotinine (4.0 g.) was refluxed for

90 minutes with concentrated hydrochloric acid (40 ml.) and water (60 ml.). At the end of this period chromic chloride hexahydrate (35 g.) was added to the hot mixture, which now contained a considerable quantity of crystalline annotinine chlorohydrin hydrochloride. Water (150 ml.), concentrated hydrochloric acid (10 ml.), and zinc powder (25 g) were also added to the mixture, which was refluxed under nitrogen for 5 hours.

The mixture was cooled, the zinc filtered out and washed with water. and the filtrate made alkaline with a large excess of ammonia with constant swirling to render the precipitated chromium salts granular. The mixture was extracted with ether in a continuous extractor and the extract evaporated to dryness. There was left an amorphous solid (4.0 g.), which was dissolved in benzene and chromatographed on alumina (Grade III). Benzene, and then ether, were used as eluants. Unsaturated lactone A (2.03 g.) m.p. 132°, and hydroxylactone (1.1 g.), m.p. 174°, were obtained from the eluants (cf. 7).

Annotininic Acid

Annotinine (1.0 g.) was refluxed for 40 minutes with barium hydroxide octahydrate (6.0 g.) and water (30 ml.). The alkaloid was completely dissolved in *ca.* 30 minutes. The solution was cooled and an excess of carbon dioxide was bubbled through it to precipitate the barium. The barium carbonate was filtered and washed well with water. The combined filtrate and washings was evaporated to dryness under reduced pressure, and the white solid residue was dissolved in methanol. This solution was concentrated on the steam bath until it began to deposit crystals and was then allowed to cool. Colorless, glistening prisms (500 mg.) were obtained by filtration, and a further crop (275 mg.) by concentration of the mother liquor. After recrystallization from methanol, the product consisted of hard glistening prisms, becoming powdery on exposure to air, m.p. 258–259° (dec.). Found: C, 65.80; H, 7.76; N, 4.57. Calc. for $C_{16}H_{23}O_4N$: C, 65.51; H, 7.90; N, 4.78%. The acid is nearly insoluble in ether, sparingly soluble in ethanol, and readily soluble in methanol and in water. The infrared absorption spectrum showed a diffuse peak at 1575–1568 $cm.^{-1}$ typical of an ionic carboxylic group and a strong hydroxyl peak at 3330 $cm.^{-1}$. Surprisingly, there was a small but well-defined peak at 3020 $cm.^{-1}$ suggestive of a double bond. No uptake of hydrogen was observed, however, when the acid in aqueous acetic acid was treated with hydrogen in the presence of Adams' catalyst. The ultraviolet spectrum of the acid in water showed continuous transmission from 220 mμ to 350 mμ. Attempts to decarboxylate the acid failed.

Annotinine Chlorohydrin from Annotininic Acid

Annotininic acid (200 mg.) was refluxed with concentrated hydrochloric acid (3 ml.) and water (4 ml.) for 90 minutes. After the solution had cooled, addition of a seed crystal of annotinine chlorohydrin hydrochloride initiated crystallization. The chlorohydrin was liberated from the hydrochloride (190 mg.) by the addition of ammonia to its aqueous solution. Annotinine chlorohydrin thus obtained melted at 190° either alone or in admixture with an authentic specimen.

Methyl Annotininate

Finely ground annotininic acid (800 mg.) was added to an ethereal solution of diazomethane. Very little reaction was apparent, but on addition of a little methanol there was a vigorous evolution of nitrogen and nearly all the acid dissolved. The reaction ceased after 10 minutes. A little unchanged material was removed by filtration and the filtrate was evaporated to dryness. The residual colorless oil was digested with several

portions of ether and the combined ether extracts concentrated until crystallization began. The ester separated as hard rectangular colorless prisms, m.p. 132–134°, 604 mg. After one recrystallization from ether, m.p. 132.5–134°. Found: C, 66.49; H, 8.30. Calc. for $C_{17}H_{25}O_4N$: C, 66.42; H, 8.20%. The infrared absorption spectrum of the ester in a nujol mull contained a hydroxyl band at 3460 cm.$^{-1}$ and an ester peak at 1732 cm.$^{-1}$. In carbon disulphide solution, the hydroxyl peak had a lower intensity and was at 3580 cm.$^{-1}$ while the ester carbonyl peak was broadened but still occurred at 1732 cm.$^{-1}$.

The methyl ester, on saponification by refluxing with aqueous barium hydroxide for 35 minutes, regenerated annotininic acid, m.p. 256–258°.

Methyl Epiannotininate

(*a*) *From Methyl Annotininate*

Methyl annotininate (90 mg.) was dissolved in a solution of potassium (100 mg.) in methanol (20 ml.) and allowed to stand at room temperature for 2 days. The solvent was then evaporated under reduced pressure at room temperature and the residue extracted with ether. When concentrated, the ethereal solution deposited a crystalline material, m.p. 163–164°, either alone or in admixture with methyl epiannotininate prepared as described below.

(*b*) *From Annotinine*

Annotinine (900 mg.) was added to a solution of potassium (1 g.) in absolute methanol (30 ml.). The solution in a stoppered flask was allowed to stand at room temperature for 72 hours; it was then evaporated in an air stream. The residue was washed with a little water and dissolved in ether. The ethereal solution was dried over sodium sulphate and concentrated. It deposited colorless pyramidal prisms, wt. 765 mg. A sample was recrystallized from ether for analysis, m.p. 163–164°. Found: C, 66.64; H, 8.37. Calc. for $C_{17}H_{25}O_4N$: C, 66.42; H, 8.20%. The infrared absorption spectrum in nujol mull showed a sharp hydroxyl band at 3510 cm.$^{-1}$ with an ester carbonyl peak at 1725 cm.$^{-1}$. In carbon disulphide solution, the hydroxyl peak is greatly diminished in intensity and occurs at 3580 cm.$^{-1}$, while the ester carbonyl peak shows a shoulder at 1738 cm.$^{-1}$ and is shifted at 1733 cm.$^{-1}$.

Annotinine Hydrate from Methyl Epiannotininate

(*a*) *By the Action of Acetic Acid*

Methyl epiannotininate (300 mg.) was dissolved in acetic acid (10 ml.) and the solution allowed to stand at room temperature for 24 hours. The solvent was evaporated under reduced pressure and the residual oil crystallized from ether, m.p. 220–224°, wt. 270 mg. Mixing with an authentic specimen of annotinine hydrate did not depress the melting point.

(*b*) *By the Action of Barium Hydroxide*

Methyl epiannotininate (200 mg.) was refluxed for 40 minutes with barium hydroxide octahydrate (1 g.) in water (15 ml.). The solution was cooled and carbon dioxide bubbled into the solution to precipitate all the barium. Barium carbonate was filtered off and the clear filtrate evaporated to dryness under reduced pressure. The residue, on crystallization from ethanol, yielded colorless hard prisms, wt. 147 mg., m.p. 227°, either alone or mixed with annotinine hydrate.

Trihydroxy Acid

Annotinine (1 g.) was refluxed for 18 hours with barium hydroxide octahydrate (6.0 g.) in water (30 ml.). To the cooled solution dilute sulphuric acid was cautiously added until precipitation of the barium sulphate was complete. The mixture was filtered and the filtrate evaporated to dryness under reduced pressure. The residual gum was extremely soluble in water and methanol, soluble in ethanol, but insoluble in acetone, ether, and ethyl acetate. Passage of an aqueous solution of the gummy sulphate through an amberlite 400-OH column failed to yield the trihydroxy acid in solid form. It was crystallized as the sulphate from absolute ethanol. The sulphate was extremely hygroscopic and, consequently, the analytical figures obtained were slightly outside the experimental limits usually accepted. Found: C, 52.80; H, 7.51. Calc. for $(C_{16}H_{25}O_5N)_2.H_2SO_4$: C, 53.32; H, 7.27%.

Annotinine Chlorohydrin from the Trihydroxy Acid

The trihydroxy acid sulphate (800 mg.) was refluxed for 90 minutes with concentrated hydrochloric acid (12 ml.) and water (16 ml.). The solution initially went bright yellow but as the reaction proceeded the intensity of the color lessened considerably. The solution was concentrated to *ca.* half volume and neutralized with aqueous sodium carbonate. This produced a precipitate that was removed by extraction with ether. The material obtained by evaporation of the ether extract was dissolved in benzene and chromatographed on alumina (Grade IV). Elution with benzene gave annotinine chlorohydrin (520 mg.) identical (m.p., mixed m.p., and I.R. spectra) with an authentic sample. Further elution with chloroform gave an oil (170 mg.) that could not be induced to crystallize.

Annotinine Diol

(*a*) *From the Trihydroxy Acid*

The trihydroxy acid sulphate (1.0 g.) was refluxed with 25% sulphuric acid (30 ml.) for 3 hours. The solution was then made strongly basic with ammonia and extracted with ether in a continuous extractor for 36 hours. Evaporation of the ether extract left a residual, slightly yellow, oil which was dissolved in methanol; the solution was treated with norite and filtered. To the filtrate 2 drops of concentrated hydrochloric acid were added, and the solution diluted with ether. The crystalline hydrochloride that separated was recrystallized several times from methanol–ether, m.p. 231–233° (dec.). Found: C, 56.69; H, 7.59. Calc. for $C_{16}H_{23}O_4N.HCl.\frac{1}{2}H_2O$: C, 56.71; H, 7.44%. The anhydrous salt melted at 295° (see below).

The perchlorate of annotinine diol was prepared from the hydrochloride by treating a methanolic solution of the latter with perchloric acid and diluting with ether. The perchlorate that separated was recrystallized from ethanol–ether, m.p. 243–244°. Found: C, 48.79; H, 6.14. Calc. for $C_{16}H_{23}O_4N.HClO_4$: C, 48.62; H, 6.06%.

(*b*) *From Annotinine*

Annotinine (1.0 g.) in 25% sulphuric acid (5 ml.) was refluxed gently for 25 hours. The cooled solution was made basic with ammonia and extracted with chloroform. The chloroform extract was concentrated and allowed to stand. It deposited a crystalline substance (261 mg.), which after recrystallization from chloroform melted at 192–193°. Its infrared absorption spectrum showed strong hydroxyl absorption at 3440 $cm.^{-1}$ and a

lactone carbonyl band at 1750 cm.$^{-1}$. Found: C, 65.68; H, 7.89; N, 4.79. Calc. for $C_{16}H_{23}O_4N$: C, 65.51; H, 7.90; N, 4.78%.

A sample of the crystalline annotinine diol thus obtained was converted to the hydrochloride, which was recrystallized from methanol–ether. The colorless crystals melted at 295–296°, either alone or mixed with the anhydrous hydrochloride (m.p. 295°) mentioned above. Found: C, 56.15; H, 7.03. Calc. for $C_{16}H_{23}O_4N.HCl$: C, 58.27; H, 7.33%. This salt on exposure to air became hydrated and then melted at 231–233° as the salt already described.

The chloroform mother liquor from which the annotinine diol had crystallized was evaporated to dryness and the residual dark tar (650 mg.) dissolved in benzene and chromatographed on alumina (grade IV). Benzene and chloroform were used as eluants. The first eluates yielded 80 mg. of colorless prisms, m.p. 227–228°, either alone or in admixture with authentic annotinine hydrate. A mixture of annotinine hydrate and annotinine diol melted at 169–177°. The remaining eluates yielded a reddish oil (510 mg.); this was dissolved in methanol and the solution neutralized with a few drops of hydrochloric acid. After the addition of a little ether the solution deposited colorless needles, m.p. 294–296°, identical with that of annotinine diol hydrochloride. The yield of annotinine hydrate was 8%, that of annotinine diol 67%.

Annotinine diol on being heated with acetic anhydride and fused sodium acetate formed a diacetyl derivative, m.p. 165–167°, as previously reported (4). Found: C, 63.81; H, 7.12. Calc. for $C_{20}H_{27}O_6N$: C, 63.64; H, 7.21%.

Oxidation of Annotinine Diol

Annotinine diol (536 mg.) in aqueous solution was oxidized with barium permanganate (984 mg.). At the end of the reaction the excess permanganate was reduced by the addition of a little alcohol. The manganese dioxide was filtered and washed repeatedly with water; it was suspended in water and dissolved by passing sulphur dioxide into the solution. Both this solution and the filtrate were worked up separately. After the barium had been removed with sulphuric acid, the product of the oxidation was isolated as the sulphate, m.p. 233–235° (dec.), either alone or in admixture with the amino acid sulphate obtained previously by oxidation of "unsaturated lactone A" (1), wt. 291 mg. The two sulphates were also identified by comparison of their infrared absorption spectra. Methylation of the acid with diazomethane gave rise to the methyl ester, m.p. 124° (4). Acetylation of the methyl ester with acetic anhydride and fused sodium acetate gave a neutral N-acetyl ester, m.p. 135°. Found: C, 63.01; H, 6.74. Calc. for $C_{17}H_{23}O_5N$: C, 63.53; H, 7.21%.

Methanolysis of the Methyl Ester of the Amino Acid

The methyl ester (354 mg.) was dissolved in anhydrous methanol (18 ml.), and potassium (50 mg.) dissolved in methanol (1 ml.) was added. The solution was kept in the dark at room temperature for 3.5 days. The solvent was then evaporated in a stream of air, the residual yellow oil dissolved in water (5 ml.), and the solution extracted with ether, acidified with sulphuric acid, and extracted with chloroform. The acid solution was alkalized with sodium carbonate and extracted with ether. This last ether extract on evaporation left a clear oil, 98 mg., that a paper chromatogram showed to be homogeneous. It was chromatographed on alumina (Grade IV) and eluted with ether–benzene. It was then distilled, b.p. 115–122° at 10^{-4} mm. Found: C, 62.12; H, 8.12. Calc. for $C_{16}H_{25}O_5N$: C, 61.71; H, 8.09%.

Treatment of Annotinine Lactam with Potassium Methoxide in Methanol

Annotinine lactam (640 mg.) was allowed to stand at room temperature for 3 days with a solution of potassium metal (1 g.) in methanol (25 ml.). The solvent was removed by evaporation in a stream of air, and the residue extracted with ether. The ethereal solution on evaporation left a colorless oil that slowly crystallized. Recrystallized from ether, it consisted of colorless prisms (30 mg.), m.p. 192–193°. Found: C, 63.53; H, 7.21. Calc. for $C_{17}H_{23}O_5N$: C, 63.40; H, 7.22%. The infrared absorption spectrum showed a sharp hydroxyl peak at 3375 cm.$^{-1}$, an ester carbonyl peak at 1723 cm.$^{-1}$, and a lactam carbonyl peak at 1636 cm.$^{-1}$.

The residue, which was not soluble in ether, was dissolved in water, and the solution was acidified with hydrochloric acid and allowed to stand a week at room temperature. It was then extracted continuously with ether for 36 hours. The extract, on evaporation, yielded a gum which after several crystallizations from water consisted of colorless prisms (380 mg.), m.p. 246–248°. Found: C, 62.75; H, 7.09. Calc. for $C_{16}H_{21}O_5N$: C, 62.62; H, 6.88%.

Oxidation of Methyl Epiannotininate

The ester (150 mg.) was oxidized with chromic oxide (100 mg.) in pyridine at room temperature for 16 hours. It yielded a product which, after recrystallization from ether, consisted of colorless prisms, m.p. 192–193° either alone or in admixture with the methyl epiester of annotinine lactam described above.

ACKNOWLEDGMENT

We wish to express our gratitude to Mr. R. Lauzon of these laboratories for preparing all the infrared absorption spectra.

REFERENCES

1. ANET, F. A. L. and MARION, L. Can. J. Chem. **33**, 849 (1955).
2. BANKIEWICZ, C., HENDERSON, D. R., STONNER, F. W., VALENTA, Z., and WIESNER, K. Chemistry & Industry, 1068 (1954).
3. BENSTEAD, J. C., GEE, R., JOHNS, R. B., MARTIN-SMITH, M., and SLATER, S. N. J. Chem. Soc. 2292 (1952).
4. HENDERSON, D. R., STONNER, F. W., VALENTA, Z., and WIESNER, K. Chemistry & Industry, 544, 852 (1954).
5. MACLEAN, D. B. and PRIME, H. C. Can. J. Chem. **31**, 543 (1953).
6. MANSKE, R. H. and MARION, L. J. Am. Chem. Soc. **69**, 2126 (1947).
7. MEIER, H. L., MEISTER, P. D., and MARION, L. Can. J. Chem. **32**, 268 (1954).
8. VALENTA, Z., STONNER, F. W., BANKIEWICZ, C., and WIESNER, K. J. Am. Chem. Soc. **78**, 2867 (1956).
9. VALENTA, Z., WIESNER, K., BANKIEWICZ, C., HENDERSON, D. R., and LITTLE, J. S. Chemistry & Industry, R40 (1956).
10. WIESNER, K., VALENTA, Z., and BANKIEWICZ, C. Chemistry & Industry, R41 (1956).
11. WIESNER, K., VALENTA, Z., AYER, W. A., and BANKIEWICZ, C. Chemistry & Industry, 1019 (1956).

AN AMPEROMETRIC TITRATION OF TITANIUM[1]

R. P. GRAHAM AND E. VANDALEN[2]

A few years ago it was shown (1) that *m*-nitrophenylarsonic acid is suited to the amperometric titration of thorium(IV), zirconium(IV), tin(IV), and uranium (as uranyl ion). With titanium(IV), however, the results were non-reproduceable under all the conditions investigated, and the conclusion was drawn that an amperometric determination of titanium by means of this arsonic acid is not feasible. Recent work in this laboratory (3) on the reaction of *m*-nitrophenylarsonic acid (MNPAA) with titanium (IV) has led to knowledge of conditions under which the amperometric titration of titanium with this reagent is possible.

Precipitation of Titanium(IV) with MNPAA

For titanium to be precipitated quantitatively as a *m*-nitrophenylarsonate the pH of the solution must be controlled carefully. If the pH is unduly low, titanium is not precipitated quantitatively or even not at all; if the pH is unduly high, titanium (though it still may be precipitated quantitatively) is in part precipitated as the hydrous oxide or as a basic salt. Moreover, the nature and concentration of the anions in the solution have an important bearing on the choice of the proper pH for precipitation, as the data of Table I show.

TABLE I

EFFECT OF ANIONS ON THE OPTIMUM pH FOR THE PRECIPITATION OF TITANIUM *m*-NITROPHENYLARSONATE (Ti ~ 0.04 MG./ML.)

Anion	Concentration (*M*)	Optimum pH*
Chloride	35×10^{-3}	1.2
Perchlorate	24×10^{-2}	0.80–1.60
Sulphate	1.1×10^{-3}	1.20–1.50
	4.1×10^{-3}	1.62–1.80
	36×10^{-3}	2.25–2.35

**During precipitation, as would be expected, the pH fell slightly from the preadjusted value of the titanium and reagent solutions (adjusted to the same desired value before mixing). The pH was recorded as the mean of the "before" and "after" values, which differed by 0.1 pH units or less. Measurements were made with a Beckman Model G pH meter.*

The greater ease with which titanium precipitates in a perchlorate medium, as compared to a sulphate medium, can be associated with the lesser tendency of perchlorate to complex with titanium. The complexing effect of sulphate is reflected, too, in the dependence of the degree of completeness of the precipitation on the concentration of sulphate in the solution. Absorptiometric tests on the filtrates using a Tiron method (4) showed that, although titanium is quantitatively (>99.9%) precipitated throughout

[1]*This work was supported by a grant from the Defence Research Board of Canada.*
[2]*Present address: Canadian Industries Limited, Millhaven, Ontario.*

the pH ranges given in Table I for the three concentrations of sulphate, the precipitation is more complete the lower the concentration of sulphate.

The range of pH over which a fixed amount of titanium reacts with a constant amount of MNPAA (Table I) was determined by analyzing, for excess MNPAA, the filtrates and washings from the filtered precipitates by a polarographic method described elsewhere (3), or by a related method in which the filtered and washed precipitates were dissolved.

From these findings the following procedure for the amperometric titration of titanium with MNPAA was developed.

Procedure

About 35 ml. of a titanium solution, containing 5 to 20 mg. of titanium in the presence of chloride and perchlorate, was put into a titration cell. This consisted of a beaker into which dipped a potassium chloride – agar bridge connected to a saturated calomel reference electrode, a bubbling tube through which nitrogen could be led to the bottom of the beaker, and a dropping-mercury electrode. The pH of the solution was adjusted to 1.2 ± 0.1 by the careful addition of 2.5 *M* ammonia or 2.5 *M* perchloric acid. Five milliliters of a 2.0 *M* solution of sodium perchlorate, which had been adjusted to a pH of 1.2 with perchloric acid, was added as supporting electrolyte. This solution also contained methyl red (at a concentration of 10 mg./l.), which functions as a maximum suppressor. The titrant was an aqueous solution of MNPAA prepared by dissolving the reagent (Distillation Products Industries, Rochester, N.Y.) in water to make a 0.8% solution, adjusting the pH to 1.2 with 60% perchloric acid, and, after 24 hours, filtering the solution.

Oxygen-free nitrogen was bubbled through the solution for 5 minutes before the first addition of titrant. After each addition of titrant (from a 10-ml. burette graduated to 0.05 ml.) the solution was stirred, by passing nitrogen through it, for 3 minutes if the equivalence point had not been reached or for 1 minute if the equivalence point had been passed. (Experiments had shown that these waiting periods were sufficient to allow for substantially complete reaction.) After each bubbling, an additional 1-minute period was allowed for the re-establishment of quiet solution conditions before the galvanometer

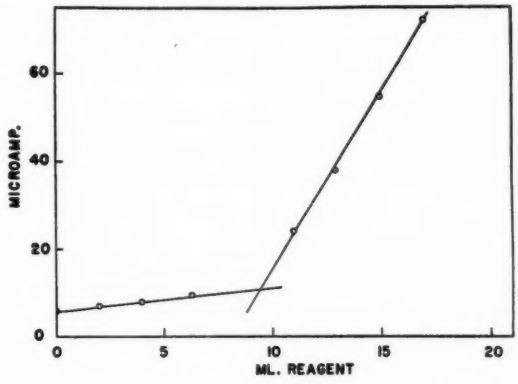


FIG. 1. Typical amperometric titration curve of titanium (0.0065 *M*), in a sodium perchlorate – perchloric acid medium of pH 1.2, with *m*-nitrophenylarsonic acid (~0.032 *M*) at pH 1.2.

deflection was recorded. The deflection, at an applied potential of −0.70 volts* vs. S.C.E., was read from the visual scale of a Sargent Model XI polarograph. Four or five readings on each side of the equivalence point were taken and the data, after correction for the dilution effect, were plotted. Fig. 1 is a typical titration graph.

The results of some titrations are shown in Table II. Multiplicate determinations had a precision of about ±0.5%; over a fourfold spread in titanium concentration the average deviation from the mean value was ±0.6%. These results are very satisfactory

TABLE II

RESULTS OF AMPEROMETRIC TITRATIONS

Titanium taken (mg.)	Volume of titrant (ml.)	Ml. of titrant per mg. of Ti
5.22	3.20	0.613
10.44	6.33*	0.606
15.66	9.46†	0.604
20.88	12.75	0.611

*Average of three determinations: 6.35, 6.28, 6.35.
†Average of four determinations: 9.48, 9.42, 9.50, 9.43.

for an amperometric titration in this concentration range (~0.005 *M*). Nevertheless, the method has rather limited practical application because of the restrictiveness of the conditions under which satisfactory titrations may be performed.

Composition of Titanium m-Nitrophenylarsonate

We have analyzed precipitates of titanium *m*-nitrophenylarsonate obtained by bringing together, under conditions similar to those obtaining in the amperometric titration described above, solutions of titanium and MNPAA in amounts corresponding to those prevailing both very close to the equivalence point and somewhat beyond it. The precipitates were filtered, washed appropriately, and dissolved in a 0.35 *M* solution of sodium tartrate. The solutions were analyzed polarographically (3) for MNPAA using a Sargent Model XX polarograph and absorptiometrically for titanium, with hydrogen peroxide, using a Coleman Model 11 spectrophotometer. The MNPAA for the calibration was obtained by purifying the commercial product, referred to earlier, by the method of (2) and the standard titanium solutions were prepared from titanium dioxide supplied by the National Bureau of Standards, the solutions being analyzed by a standard gravimetric procedure.

The average of analyses of eight precipitates was 0.94 moles of reagent for every mole of titanium. Thus, under these conditions of precipitation, a monoarsonate is formed and the reaction is probably, as suggested by Kolthoff and Johnson (1),

$$TiO^{++} + H_2R \rightarrow TiOR + 2H^+$$

where H_2R is MNPAA, $NO_2C_6H_4AsO(OH)_2$.

When, however, a very considerable excess of reagent is present, a diarsonate is formed. This was demonstrated by experiments in which known amounts of titanium in a perchlorate medium were precipitated by MNPAA at pH 1.2 under conditions that had been shown to precipitate the titanium quantitatively, and with about 20 moles of reagent present for each mole of titanium. The precipitates were dissolved as before and

*Subsequent work indicated that −0.50 volts would have been a more suitable setting.

analyzed polarographically for MNPAA, the calibration being done using a purified sample of the reagent. The average value from seven determinations of the number of moles of reagent per mole of titanium was 2.02 (with an average deviation of ± 0.03 from this average). It is clear that when the monoarsonate reacts with excess reagent a diarsonate, TiR_2, is formed and not a compound of formula $TiR_{1.5}$, as has been suggested (1).

1. KOLTHOFF, I. M. and JOHNSON, R. A. J. Electrochem. Soc. **98**, 138 (1951).
2. RUDDY, A. W. and STARKEY, E. B. Organic syntheses. Collective Vol. III. John Wiley and Sons, Inc., New York. 1955. p. 665.
3. VANDALEN, E. and GRAHAM, R. P. Anal. Chim. Acta, **12**, 489 (1955).
4. YOE, J. H. and ARMSTRONG, A. R. Anal. Chem. **19**, 100 (1947).

RECEIVED DECEMBER 7, 1956.
BURKE CHEMICAL LABORATORIES,
HAMILTON COLLEGE, MCMASTER UNIVERSITY,
HAMILTON, ONTARIO.

CANADIAN JOURNAL OF CHEMISTRY

Notes to Contributors

Manuscripts

(i) ***General.*** Manuscripts, in English or French, should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts and illustrations should be carefully checked before they are submitted. Authors will be charged for unnecessary deviations from the usual format and for changes made in the proof that are considered excessive or unnecessary.

(ii) ***Abstract.*** An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References.*** References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should not be given and only initial page numbers are required. The names of periodicals should be abbreviated in the form given in the most recent *List of Periodicals Abstracted by Chemical Abstracts*. All citations should be checked with the original articles and each one referred to in the text by the key number.

(iv) ***Tables.*** Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Vertical rules should be used only when they are essential. Numerous small tables should be avoided.

Illustrations

(i) ***General.*** All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line Drawings.*** Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used unless it is desired to have all the co-ordinate lines show. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut of suitable size.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. In large drawings or groups of drawings the ratio of height to width should conform to that of a journal page but the height should be adjusted to make allowance for the caption.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs.*** Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard with no space or only a very small space (less than 1 mm.) between them. In mounting, full use of the space available should be made (to reduce the number of cuts required) and the ratio of height to width should approximate that of a journal page (5⅛ × 7⅞ in.); however, allowance must be made for the captions. Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.5 the number of manuscript pages (double-spaced typewritten sheets, 8½ × 11 in.) and including the space occupied by illustrations. An additional charge is made for illustrations that appear as coated inserts. The cost per page is given on the reprint requisition which accompanies the galley.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

Contents

Page

Kinetics of the Thermal Decomposition of Hydrogen Peroxide Vapor—*Paul A. Giguère and I. D. Liu* - - - - - - - - - - - - - - - - - 283

Surface Studies Using Ion Exchange Autochromatography—*Ruth E. Krehbiel and J. W. T. Spinks* - - - - - - - - - - - - - - - - - 294

The Olefinic Bond in Gelsemine—*Léo Marion and K. Sargeant* - - - - - 301

Free Radicals by Mass Spectrometry. XII. Primary Steps in the Mercury Photosensitized Decompositions of Acetone and Acetaldehyde—*F. P. Lossing* - 305

Hydrogenolysis of Carbohydrates. II. Reduction of Methyl α-D-Glucopyranoside—*E. von Rudloff, D. E. Stuetz, and H. F. Bauer* - - - - - - - 315

Chromatography of Waste Sulphite Liquor—*A. C. Shaw and M. Dignam* - - 322

Vegetable Ivory as a Source of a Mannan Polysaccharide—*T. E. Timell* - - 333

Internal Rotation. IX. The Infrared and Raman Spectra of Liquid Normal Alkyl Bromides—*T. Yoshino and H. J. Bernstein* - - - - - - - - - - 339

Organic Deuterium Compounds. XVI. Synthesis of α-Deuterated Alkyl Nitriles —*L. C. Leitch* - - - - - - - - - - - - - - - - - - - 345

Organic Deuterium Compounds. XVII. A Synthesis of Deuterated Methyl Chloroform—*J. E. Francis and L. C. Leitch* - - - - - - - - - - 348

Some Acenaphthene Compounds—*E. H. Charlesworth, H. Campbell, J. J. Conn, C. T. Elston, and D. L. Stachiw* - - - - - - - - - - - 351

An Oxygenated Fatty Acid from the Seed Oil of *Hibiscus esculentus*—*Mary J. Chisholm and C. Y. Hopkins* - - - - - - - - - - - - - - - 358

The Configuration of Glycosidic Linkages in Oligosaccharides. IV. Further Degradations of Reducing Disaccharides to 2-*O*-Glycosyl-glycerols—*A. J. Charlson, P. A. J. Gorin, and A. S. Perlin* - - - - - - - - - - 365

A Recording Vacuum Thermobalance—*J. G. Hooley* - - - - - - - - - 374

Viscosity of Natural Rubber Solutions at Very Low Rates of Shear—*Morton A. Golub* - 381

Structure of a Hemicellulose from Maple Wood Previously Extracted by Liquid Ammonia—*L. G. Neubauer and C. B. Purves* - - - - - - - - - - 388

Some Oxidation Reactions of Delcosine—*Ragini Anet, D. W. Clayton, and Léo Marion* - 397

The Structure of Annotinine—*M. Martin-Smith, R. Greenhalgh, and Léo Marion* - 409

Notes:

An Amperometric Titration of Titanium—*R. P. Graham and E. VanDalen* - 418

Printed in Canada *by* University of Toronto Press